TAMPERE UNIVERSITY OF TECHNOLOGY

TITTA HAGELIN

THE EFFECTS OF RIVER WATER INFILTRATION ON SUB-SURFACE MICROBIOLOGY AND PHYSICO-CHEMICAL WATER CHARACTERISTICS IN A PRISTINE AQUIFER

Master of Science Thesis

Examiners: Professor Jaakko Puhakka, M.Sc. Johanna Ojala
Examiners and topic approved in the Science and Environmental Engineering Faculty Council meeting on 11th January 2012

ABSTRACT

TAMPERE UNIVERSITY OF TECHNOLOGY
Master's Degree Programme in Environmental and Energy Technology
HAGELIN, TITTA: The effects of river water infiltration on sub-surface microbiology and physico-chemical water characteristics in a pristine aquifer
Master of Science Thesis, 91 pages
April 2012
Major: Environmental Biotechnology
Examiners: Professor Jaakko Puhakka, M.Sc. Johanna Ojala
Keywords: artificial groundwater recharge, bacterial community structure, bank filtration, biodegradation, contaminants, LH-PCR, natural organic matter

Artificial groundwater recharge (AGR) and bank filtration (BF) are old, widely applied methods to produce drinking water by the infiltration of surface water through soil. These methods are increasingly used because they are considered to offer a relatively inexpensive and sustainable way to increase the water quality by physical, chemical and biological processes. One of the primary goals of AGR and BF is the removal of natural organic matter (NOM) that has several indirect adverse effects. Pollutants possibly existing in aquatic environments may pose a risk of aquifer contamination and have harmful effects for human health. Biodegradation has an important role in sustainable removal of NOM and pollutants in AGR and BF. However, the mechanisms affecting the natural removal processes are still poorly understood, and therefore more knowledge of underlying biogeochemical processes is needed.

A research project TEVA (Improving artificial groundwater recharge by the development of on-line monitoring and control) was established to improve the understanding of the removal mechanisms of NOM during AGR and to enhance the AGR process control by on-line monitoring. As a part of the TEVA-project, the aim of this thesis was to study the effects of surface water infiltration on a pristine aquifer by monitoring physico-chemical water and bacterial community changes in Virttaankangas AGR site, southwest Finland, during experimental river water infiltration. In addition, the feasibility of biofilm collector slides for aquifer biofilm sampling was evaluated.

The progress of infiltrated water in the aquifer was evident from increased dissolved organic carbon (DOC) concentrations. Compared to the initial infiltrated water concentration, DOC removal was observed along the soil passage. However, no preferential removal of any molecular fraction of DOC could be demonstrated.

Due to infiltration, the native groundwater bacterial community profile changed significantly towards the community composition of infiltrated water. Attached bacterial community composition was not strongly influenced by water infiltration. Leucine incorporation method confirmed bacterial activity in the subsurface, whilst the RNA-based community analysis indicated that only a minor part of the attached and unattached communities were responsible for the activity in the aquifer.

Differences in the minor bacterial groups between soil and biofilm collector community compositions were observed. However, the biofilm collectors accumulated dominating soil bacterial groups and therefore the collectors reliably collected the dominant soil bacteria.

TIIVISTELMÄ

TAMPEREEN TEKNILLINEN YLIOPISTO
Ympäristö- ja energiatekniikan koulutusohjelma
HAGELIN, TITTA: Jokiveden imeytyksen vaikutus koskemattoman akviferin mikrobiologiaan ja veden fysikaalis-kemiallisiin ominaisuuksiin
Diplomityö, 91 sivua
Huhtikuu 2012
Pääaine: Ympäristöbiotekniikka
Työn tarkastajat: Professori Jaakko Puhakka, DI Johanna Ojala
Avainsanat: bakteeriyhteisön rakenne, biohajoaminen, LH-PCR, luonnollinen orgaaninen aines, saasteet, tekopohjaveden valmistus

Tekopohjaveden muodostaminen muun muassa allasimeytyksen tai rantaimeytyksen avulla on vanha, laajalti käytössä oleva menetelmä tuottaa talousvettä. Tekopohjavesiprosessissa veden laatu maaperässä paranee fysikaalisten, kemiallisten ja biologisten reaktioiden kautta, ja menetelmän hyödyntäminen talousveden tuotannossa on maailmanlaajuisesti lisääntymässä. Luonnollisen orgaanisen aineksen (natural organic matter, NOM) poistaminen on yksi tekopohjavesiprosessin päätavoitteista sen epäsuorien haittavaikutuksien takia. Raakavesiympäristössä mahdollisesti esiintyvät saasteet aiheuttavat tekopohjavesiakviferien kontaminoitumisriskin ja läpäistessään käsittelyn ne vaikuttat myös talousveden laatuun. Biohajoamisella on tärkeä rooli luonnollisen orgaanisen aineksen ja saasteiden poistamisessa tekopohjavedestä. Luonnollisten poistumismekanismien taustatekijöistä ei kuitenkaan ole vielä syvällistä tietoa, minkä takia prosessiin vaikuttavia mikrobiologisia ja kemiallisia tekijöitä on tutkittu viimeaikoina paljon.

TEVA-hanke (tekopohjaveden valmistusprosessin tehostaminen on-line monitorointia ja ohjausta kehittämällä) perustettiin luomaan lisää tietoa NOM:n poistumisesta tekopohjavesiprosessissa ja arvioimaan on-line monitoroinnin hyödyntämistä prosessien seurannassa. Osana TEVA-hanketta tämän diplomityön tavoitteena oli tutkia 1) jokiveden imeyttämisen vaikutuksia koskemattomaan akviferiin tarkkailemalla kemiallisia ja mikrobiologisia muutoksia Lounais-Suomessa sijaitsevalla Virttaankankaan imeytysalueella viiden kuukauden kokeellisen jokivesi-imeytyksen aikana, sekä 2) biofilmikeräimien soveltuvuutta akviferibiofilmien näytteenottoon.

Jokiveden eteneminen Virttaankankaan akviferissä oli selvästi havaittavissa kohonneiden liukoisen orgaanisen hiilen (dissolved organic carbon, DOC) pitoisuuksien perusteella. Maaimeytyksen havaittiin vähentävän veden DOC-pitoisuutta imeytetyn veden DOC-pitoisuuksiin verrattaessa, mutta minkään tietyn orgaanisen aineksen kokofraktion ensisijaista poistumista ei koejakson aikana tapahtunut.

Imeytysprosessi vaikutti merkittävästi luonnollisen pohjaveden vesifaasin bakteeriyhteisöjen rakenteeseen, joka muuttui jokiveden etenemisen myötä imeytettävän veden yhteisön kaltaiseksi. Maaperän partikkeleiden pinnoille kiinnittyneisiin mikrobiyhteisöihin imeytyksellä ei ollut vaikutusta. Tekopohjavesiakviferin mikrobien aktiivisuus osoitettiin bakteerisolujen leusiinin inkorporaatiomäärityksin. RNA-perusteisen mikrobiyhteisöanalyysin mukaan vain vähäinen osa vesifaasin bakteereista ja kiinnittyneistä bakteereista olivat aktiivisia. Biofilmikeräimet kuvasivat luotettavasti maa-ainekseen sitoutuvat dominoivat bakteeriryhmät.

PREFACE

The study for this thesis was carried out at the Department of Chemistry and Bioengineering of Tampere University of Technology in the Laboratory of Environmental Engineering and Biotechnology, and funded by the Finnish Funding Agency for Technology and Innovation, Turku Region Water Ltd, Tavase Ltd, Pöyry Environment Ltd, and FCG Planeko Ltd.

I would like to thank my supervisor Professor Jaakko Puhakka for offering me the chance to participate this interesting project and for his comments and advisement during this study. I am very grateful to my other supervisor, M.Sc. Johanna Ojala for all her practical guidance, help and support with the laboratory analyses and samplings. I would also like to thank her for performing the activity analyses presented in this thesis. I want to thank Dr. Kathryn Wakeman for her guidance and advices for microbial laboratory studies. The personnel of Turku Region Water Ltd. are thanked for their help in the samplings and for sharing their information.

My warmest thanks I would like to dedicate for my family and friends, and especially for my fiancé Daniel for all the encouragement, support and love during my studies. It has meant a lot to me.

Berlin, 12th of March 2012

Titta Hagelin

CONTENTS

ABBREVIATIONS

AGR	artificial groundwater recharge
AOC	assimilable organic carbon
AOI	adsorbable organic iodine
BDOC	biodegradable dissolved organic carbon
BF	bank filtration
bp	base pairs
DAPI	4',6-diamidino-2-phenylindole
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
EDC	endorcine disrupting compound
HDPE	high density polyethylene
HPLC	high pressure liquid chromatography
HPSEC	high pressure size exclusion chromatography
LH-PCR	length heterogeneity – polymerase chain reaction
MAR	managed aquifer recharge
MW	molecular weight
NOM	natural organic matter
NSAID	non-steroidal anti-inflammatory drug
PCP	personal care product
PCR	polymerase chain reaction
PhAC	pharmaceutically active compound
POM	particulate organic matter
RNA	ribonucleic acid
RT	reverse transcription
SDS	sodium dodecyl sulphate
SEC	size exclusion chromatography
SUVA	specific UV-absorbance
TOC	total organic carbon
UVA	ultraviolet absorbance

1. INTRODUCTION

Managed aquifer recharge (MAR) has been used to produce drinking water since the end of 19th century in Europe and in the USA. In MAR processes such as artificial groundwater recharge (AGR) and bank filtration (BF), surface water is infiltrated into an aquifer, where organic and inorganic compounds are partly removed by physical, chemical and biological processes during sub-surface flow. (Huismans and Wood 1974) Compared to conventional and advanced drinking water treatment, this natural water treatment process has advantages, including low costs, low energy requirements, and minimal chemical consumption and waste stream production (Hoppe-Jones et al. 2010).

In drinking water, natural organic matter (NOM) causes taste, odour, and colour, produces disinfection by-products, and increases bacterial regrowth in water distribution systems (Myllykangas et al. 2002; Leenheer and Croué 2003). Therefore one of the primary goals of MAR is the reduction of NOM to levels that will not cause undesired effects. In areas with dense population, water affected by municipal and industrial sewage is often used indirectly for potable use via MAR (Heberer et al. 2008). Since the analytical methods enabled detection of trace organic chemicals in aquatic environments, an increased concern about the risk of pollutants in MAR processes has awoken. Pollutants in raw water source may contaminate aquifers used for MAR and have adverse effects for human health, and therefore their attenuation during MAR should be ensured. (Díaz-Cruz and Barceló 2008; Maeng et al. 2011a)

Although the long operation time of many MAR systems provides empirical evidence for sustained removal or transformations of organic matter (Fox et al 2005), the actual removal mechanisms of NOM and organic pollutants during MAR processes are not fully understood. Biodegradation has a major role in sustainable NOM and pollutant removal (Díaz-Cruz and Barceló 2008). However, little is known about the role of microbial ecology and microorganisms, and factors like redox conditions, temperature, organic matter characteristics and retention time in MAR processes. Recently many studies have been performed to characterize different removal processes and factors affecting them (e.g. Kortelainen and Karhu 2006; Kolehmainen 2008; Groß-Wittke et al. 2010; Hoffmann and Gunkel 2011b; Maeng et al. 2011b). Insufficient removal of some compounds during MAR has increased interest also in MAR process optimization research (Zhu et al. 2010; Grützmacher and Reuleaux 2011).

This thesis is a part of a TEVA-research project (Tekopohjaveden valmistusprosessin tehostaminen on-line monitorointia ja ohjausta kehittämällä; Improving artificial groundwater recharge by the development of on-line monitoring and control). The aim of the project was to improve the understanding of removal mechanisms of NOM during AGR and to enhance the AGR process control by on-line monitoring. The project was coordinated by the Department of Chemistry and Bioengineering of Tampere University of Technology, and realised in cooperation with the Universities of Helsinki and Jyväskylä, and several enterprises.

The TEVA-project included monitoring of a full-scale AGR plant Jäniksenlinna in Tuusula, and two planned recharge sites: Virttaankangas in Alastaro and Oripää, and Syrjänharju in Pälkäne. The aim of this thesis was to 1) study the effects of river water infiltration in a pristine aquifer by monitoring the associated physico-chemical and microbial changes in Virttaankangas AGR site, and 2) to evaluate the feasibility of biofilm collector slides for aquifer biofilm sampling. The start-up of a new AGR plant in Virttaankangas offered a unique possibility to monitor changes in a pristine aquifer at field scale.

This thesis comprises a literature review and an experimental part. In the literature review, the fundamentals of biological drinking water production and managed aquifer recharge processes are described. Biological NOM removal, factors affecting the removal, and changes in NOM characteristics during MAR are reviewed, as well as the risks of pollutants in MAR processes. Attenuation of iron, manganese, cyanotoxins, pathogens, pharmaceuticals and trace organic compounds in MAR are described, followed by process optimization options for biofilters and especially for MAR processes. The literature review is followed by the experimental part of this thesis. Virttaankangas AGR site, and the chemical and microbial monitoring methods and assays are described in section 3. Results are presented and discussed in section 4, followed by conclusions of the experimental work in section 5.

2. THEORY

2.1. Biological drinking water production

Biological drinking water production can be used to purify many organic and inorganic contaminants or compounds in surface water and in groundwater. These compounds and pollutants include, among others, natural organic matter, iron, manganese, several pollutants from industrial, municipal, and agricultural activities, as well as pathogens. Biological treatment processes are based on microbial communities capable of metabolizing contaminants from a water source. In biodegradation processes bacteria gain energy and reproduce by mediating the transfer of electrons from reduced compounds to oxidized compounds. Heterotrophs use organic compounds as their electron donors, while autotrophs utilize inorganic compounds. (Huismans and Wood 1974; Brown 2007; Zhu et al. 2010) Biological drinking water treatment decreases the potential for bacterial regrowth, reduces the formation of chlorinated disinfection by-products and the demand of chlorine, and decreases corrosion potential. In addition, taste, colour, and odour-causing compounds and micropollutants of health concern can be controlled. (Urfer et al. 1997)

Biological drinking water production processes are mostly fixed biofilm systems, where bacterial communities are developed on a media such as sand, anthracite, granular activated carbon, or membranes. In biological active filters biofilms develop on a stationary bed of media, whereas in fluidized-bed processes granular media is used to support the development of biofilms. A small number of biological treatment processes are based on suspended growth systems. Even though biological active filters have been used for over two centuries to produce drinking water, they have gained more attention only recently. Reasons for this are, among others, the rising costs and complexities of handling water treatment residuals, regulations to limit the formation of disinfection by-products, the push for green technologies and remediation methods, and the need of safe and sustainable water sources and storages. (Bouwer 2000; Brown 2007; Zhu et al. 2010)

In its simplest form, biological drinking water treatment includes the filtration of water through a bed of sand, a process called slow sand filtration. Groundwater has been accepted as the purest source for domestic purposes for many centuries, but with the growth of industry and population, many groundwater sources were insufficient to meet the demand. The search for extra water sources led to augmentation of aquifers by managed aquifer recharge, which includes bank filtration and artificial groundwater

recharge. The same biological processes occurring in slow sand filtration are exploited in MAR processes. (Huismans and Wood 1974; Maeng et al. 2011b) In comparison to conventional and advanced drinking water treatment, managed aquifer recharge is a low-cost and low-energy alternative with minimal waste stream production taking advantage of natural physical, chemical, and biological attenuation processes. (Massmann et al. 2006; Hoppe-Jones et al. 2010) In the following, the basics of slow sand filtration and MAR processes are introduced.

Slow sand filtration

Slow sand filtration is the oldest drinking water treatment process and it has been used continuously since the 1800s. It is an inexpensive, simple and reliable method to produce drinking water, and numerous modifications of biological filter reactors have been developed. Conventional slow sand filtration is accomplished by passing raw water through a bed of sand where purification takes place during downward passage. The major function occurs at the highly biologically active schmutzdecke, the surface layer of the filter bed consisting of deposited and synthesized material, highly biologically active bacteria, algae, protists and macroinvertebrates (Unger and Collins 2008; Zhu et al. 2010). Figure 2.1 presents a diagram of a slow sand filter consisting of filter bed medium, a layer of graded support gravel, an underdrain system and a system of control valves. The filter bed medium generally consists of sand and is usually 1 m deep in a new filter bed. Underdrain system collects filtered water and control valves are used to regulate the velocity of flow through the bed. (Huismans and Wood 1974; Collins et al. 1992)

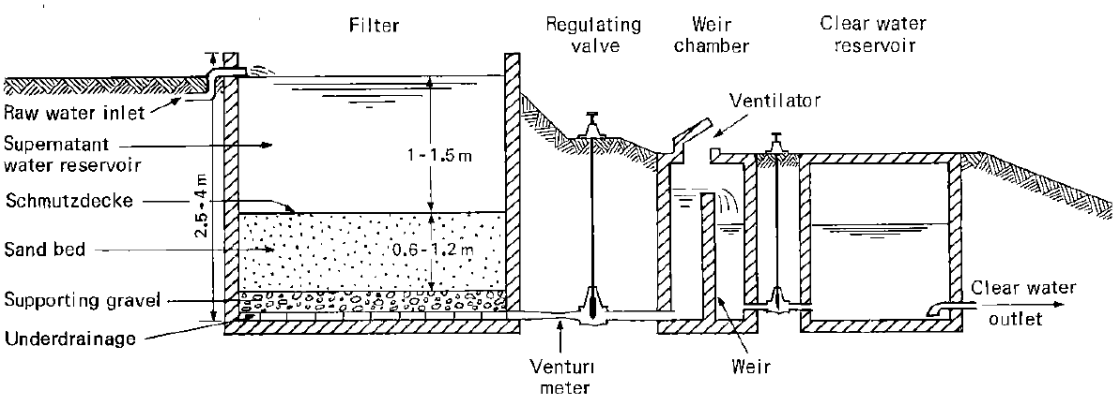

Figure 2.1. *Diagram of a slow sand filter (Modified from Huismans and Wood 1974).*

Bank filtration

Riverbank filtration has been used to produce drinking water since the 1870s and in many countries bank filtration is used to produce drinking water from alluvial aquifers hydraulically connected to a water course. Bank filtration can occur naturally or it can be induced by pumping water from vertical or horizontal wells placed along river or

lake bank in order to lower the groundwater table below the surface water level. This causes the surface water to flow through the colmation layer and the aquifer towards the wells. At the same time, attenuation of surface water contaminants takes place by microbial degradation, filtration, sorption, chemical precipitation, redox reactions, and dilution with background groundwater as presented in Figure 2.2. (Hiscock and Grischek 2002; Ray et al. 2002) Due to these processes, suspended solids, particles, biodegradable compounds, bacteria, viruses, and parasites in the water are eliminated, adsorbable compounds partly eliminated, and temperature changes and concentrations of dissolved compounds equilibrated. The attenuation of surface water contaminants in bank filtration depends strongly on site-specific hydrogeological and hydrochemical conditions. Well operated bank filtration facilities situated in favourable hydrogeological settings can provide relatively inexpensive high-quality water that needs little further treatment. (Hiscock and Grischek 2002; Grünheid et al. 2005)

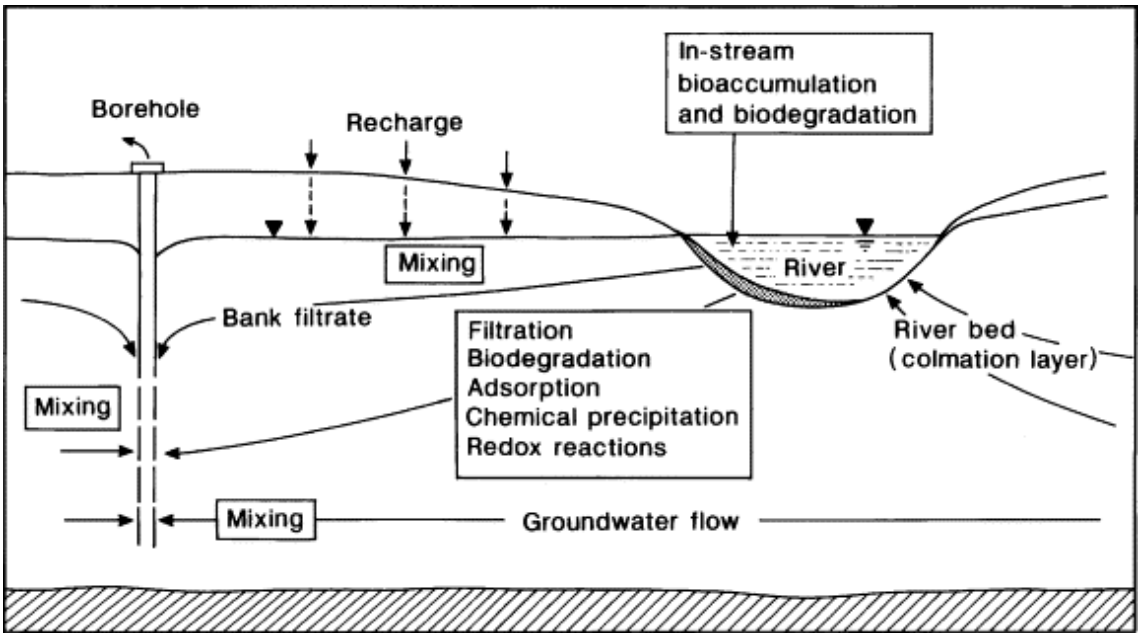

Figure 2.2. *A schematic illustration of riverbank filtration and processes affecting water quality during bank filtration (Hiscock and Grischek 2002).*

The design and operation of bank filtration sites differs depending on the treatment objectives. In Europe, bank filtration is often considered as a major part of the overall treatment and the goal is to produce high quality, biologically stable water with minimal need of additional treatment and without chlorine addition. This is achieved with retention times of several weeks or months. In North America, bank filtration has been in use less than 60 years (Ray et al. 2002) and it is considered as pre-treatment in a multiple barrier concept. Retention times ranging from several hours to a few weeks, the goal primarily is to remove pathogenic microorganisms and reduce the costs of conventional drinking water treatment. The secondary objective is the removal of turbidity and some dissolved organic carbon. (Grünheid et al. 2005)

Artificial groundwater recharge

In 1897, the first application of artificial recharge for water production was taken in use in Sweden. (Huismans and Wood 1974) Artificial recharge of aquifers has become a valuable tool to enhance water sources and it is increasingly used for water production in many countries to meet the demands for growing urban populations and in water-shortage areas. (Långmark et al. 2004; Kuster et al. 2010) Artificial recharge systems are engineered systems designed for treatment and storage of water in aquifers. Other objectives can be, among others, the reduction of seawater intrusion or land subsidence, control of ground water levels, and usage as water transportation systems. As illustrated in Figure 2.3, in artificial recharge water from external source is infiltrated through soil to recharge aquifer. On surface infiltration basins, furrows, ditches, sprinkling networks or other facilities are used to accomplish artificial recharge. Injection wells are used for water infiltration through the vadose zone or for direct injection into the aquifer. The purified water is gained from the aquifer through production wells located in sufficient distance from the infiltration areas. (Bouwer 2002)

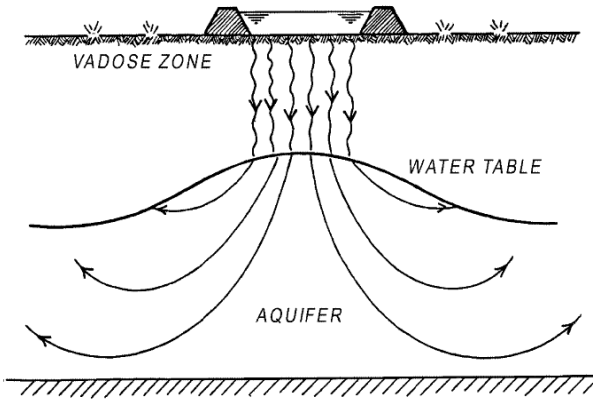

Figure 2.3. *Illustration of a typical groundwater recharge system with an infiltration basin (Bouwer 2002).*

Water used for recharge can be infiltrated without pretreatment or it can be treated to a required level to avoid undesirable effects during the process. (Huismans and Wood 1974; Sharma and Amy 2010) As well as bank filtration, artificial recharge takes advantage of natural treatment processes as the water moves through the soil and aquifer. Sorption, biodegradation, physiochemical processes and mixing are responsible for the removal of chemical pollutants and microorganisms. (Långmark et al. 2004; Maeng et al. 2011b)

2.2. Biological natural organic matter removal in MAR

2.2.1. Natural organic matter

In drinking water, natural organic matter has disadvantageous side effects by causing taste, odour, and colour. It can also bind and transport organic and inorganic contaminants, produce undesirable disinfection by-products, and increase bacterial regrowth in water distribution systems. (Myllykangas et al. 2002; Leenheer and Croué 2003) Thus, one of the primary goals of water treatment is the reduction of natural organic matter to levels that will not cause disadvantageous effects.

Natural organic matter is a term used to describe all the organic matter excluding living organisms and compounds of man-made origin. NOM can be found everywhere in natural waters where most of it occurs as dissolved organic matter (DOM) or dissolved organic carbon (DOC) (Leenheer and Croué 2003). The source of NOM can be allochthonous, originating from outside of the waterbody or a given system, or autochthonous, formed within a given system. NOM is a mixture of very complex and heterogeneous organic compounds, and different theories of its structure have been proposed. The several coexisting NOM classifications are based on the analytical approach applied in NOM analyses. (Filella 2009)

Depending on the sources, organic compounds from human activity can also be present in significant amounts in surface water and groundwater. This is often the case in source water used for infiltration in many MAR sites. These compounds are included in organic carbon measurements, for example in total organic carbon (TOC) or dissolved organic carbon that are the most common parameters used to quantify NOM in waters. (Filella 2009) Other commonly used methods for organic matter investigation and characterization in drinking water are, among others, (specific) UV-absorbance (SUVA), biodegradable dissolved organic carbon (BDOC), assimilable organic carbon (AOC), fluorescence spectroscopy, and fractionation techniques, in particular XAD resin fractionation and size exclusion chromatography (SEC) (Filella 2009; Matilainen et al. 2011).

UV-absorbance measurements are used to indicate the degree of NOM aromaticity in a sample and SUVA represents the relative aromaticity of dissolved organic matter. (Leenheer and Croué 2003) BDOC is the portion of the dissolved organic carbon in water that can be mineralized by heterotrophic microorganisms. Incubation time can last from few days up to about one month. AOC is the portion of the BDOC that can be converted to cell mass and expressed as a carbon concentration by means of a conversion factor or calibration. (Huck 1990) Fluorescence spectroscopy is used to characterize protein-like and humic-like compounds from which humic-like compounds can be further identified as fulvic and humic acids (Chen et al. 2003). XAD resin fractionation can separate NOM into humic, or hydrophobic substances and into

hydrophilic organic matter. Size exclusion chromatography separates molecules and particles according to their size distribution and molecular weight. (Page and Dillon 2007)

2.2.2. Changes in NOM characteristics during MAR

Many studies have characterized the changes in the properties of DOM during field site MAR and in lab-scale experiments with the methods described in the previous section. The SUVA measurements in several studies commonly indicate the removal of aliphatic carbon sources resulting mainly from aerobic biodegradation. (Grünheid et al. 2005; Xue et al. 2007; Xue et al. 2009; Maeng et al. 2011a) In an experiment of Xue et al. (2007) most of the UV-254 removal occurred within the first 0,5 m of the columns, and only minor decrease was observed during further percolation. With decreasing DOC values this resulted in monotonically increasing SUVA across the columns. Grünheid et al. (2005) observed increasing SUVA values at oxic bank filtration pathway but again decreasing SUVA values in anoxic soil passage. The results indicated that during anaerobic infiltration more aromatic and double-bond structures were degraded. According to the field site and lab-scale studies (Grünheid et al. 2005; Xue et al. 2007; Xue et al. 2009), it has been commonly concluded that aliphatic organic matter is preferentially removed during oxic BF and AGR (Maeng et al. 2011b). However, dissident results have also been observed, and no clear trend in aromatic carbon removal could be demonstrated in the subsurface flow path at some AGR sites (Kolehmainen et al. 2007).

Studies performed by XAD resin fractionation show that during MAR hydrophilic fraction of DOM in water is preferentially removed compared to hydrophobic organic matter or other NOM fractions. Xue et al. (2009) studied the removal of several fractions in effluent derived DOC and reported that when proportioned to the quantity of the fractions, hydrophilic acids were removed most efficiently followed by transphilic neutrals, hydrophobic neutrals, and approximately similar removal of hydrophobic acids and transphilic acids. Because hydrophilic organic matter contains a relatively high amount of aliphatic organic matter, the results conducted with XAD resin fractionation are in agreement with SUVA results confirming the suggest that aliphatic organic matter is preferentially removed. (Maeng et al. 2011b)

Size exclusion chromatography measurements in several field site MAR studies and column experiments have shown that large molecular fractions of DOM can be efficiently or totally removed, and that they are more efficiently removed compared to the smaller fractions. (Grünheid et al. 2005; Kolehmainen et al. 2007; Laws et al. 2011; Maeng et al. 2011a) The large molecular weight fraction is interpreted as a biopolymer fraction consisting of easily biodegradable, non-humic organic matter such as proteins and polysaccharides. Fractions consisting of humic substances, humic-like polymer building blocks, low molecular weight acids, and others, have been shown to be

removed but to a lesser extent. (Grünheid et al. 2005; Laws et al. 2011; Maeng et al. 2011b) Grünheid et al. (2005) showed that the fractions of DOC behave similarly under anoxic/anaerobic conditions and aerobic conditions. Under oxic conditions most changes in the DOC-fractions took place during initial infiltration and further change of remaining non-biodegradable DOC was minimal. The preferential removal of large molecular weight fraction is in agreement with the reported increase of SUVA and the preferential removal of hydrophilic organic matter (Maeng et al. 2011b).

The fluorescence spectra of different studies also confirm the results of SUVA and size exclusion chromatography measurements and the findings from resin fractionation. The study of Laws et al. (2011) showed that the protein-peak had the greatest intensity compared to the humic- and the fulvic-peaks in an AGR basin. The intensity of the three peaks decreased as water migrated through the vadose zone into the aquifer leading to strongest decrease of the protein peak. Likewise, column studies have shown better removal of the protein-like peak compared to the other humic-like peaks in effluent affected water. Xue et al. (2009) reported that sorption and anaerobic biodegradation didn't seem to have significant effect on the fluorescence properties of DOM, whereas aerobic biodegradation significantly altered the chemical structure of fluorescent constituents in DOM. It has been concluded, according to the different results, that preferential, microbial activity attributed removal of non-humic substances exists during MAR in aerobic conditions (Maeng et al. 2011b).

2.2.3. Microorganisms and NOM biodegradation in MAR

Microorganisms are found in large numbers in soil, which represents a favourable habitat for them. The density of microorganisms is highest in the top soil layer. Within the first meter of soil profiles microbial biomass declines rapidly in parallel with nutrient decline but then stabilizes. The microbial communities in deeper sediments differ from the surface populations and their composition and structure changes significantly with soil depth and resource availability. At the groundwater table the number or cells usually increases. (Kolehmainen 2008; Schütz 2008)

Microbial communities within aquifers consist largely of heterotrophs but also autotrophs are an important component of the communities. Soil and aquifer microorganisms are responsible for biodegradation of organic compounds entering the soil and therefore play an important role in the recycling of nutrients and the maintenance of groundwater quality. NOM is used as energy and carbon source in biodegradation and the final products include inorganic carbon, water, new biomass, metabolites, and excreted cellular components. (Kolehmainen 2008; Schütz 2008) Generally, TOC and DOC removal efficiencies in MAR processes range from 30 to 88% and 33 to 88%, respectively. The major biodegradation of NOM has been commonly reported to occur dominantly within the first few meters of infiltration. (Maeng et al. 2011b) However, contradictory results have also been reported, where the

major TOC concentration reduction was observed in the groundwater zone. (Helmisaari et al. 2006)

Groundwater habitats are characterised by lack of light, low availability of organic carbon and nutrients, low temperatures, and hydrological, chemical and geological heterogeneity. Although in every aquifer the vertical layering of strata is unique and possibly very complex, the environmental conditions can be very stable within different zones. Microbial communities within aquifers are expected to be well adapted to the nutrient-poor and oligotrophic groundwater environment. (Giebler and Lueders 2009)

The total number of bacteria ranges between 10^2 and 10^6 cells per cm^3 of groundwater and between 10^4 and 10^8 cells per cm^3 of sediment. The availability of DOC and nutrients, the sediment grain-size distribution, and the mineralogy of the sediments affect the ratio of suspended to attached microbes. In carbon- and nutrient-poor environments attached mode of life is advantageous and thus most prokaryotes in aquifers are attached to sediment surfaces forming microcolonies and biofilms. Surfaces are geochemically more diverse and offer more ecological niches than groundwater. (Giebler and Lueders 2009; Humphreys 2009) This leads to differing community compositions, and Flynn et al. (2008) demonstrated that attached and suspended samples from the same well shared on average only one third of their microbial community. Possible reasons for this may be that the microbes are inactive, prefer groundwater environment or they are excluded from sediment surfaces by competition from other bacteria.

It has been hypothesized that the microbial community composition of soil and aquifer determines its potential for substrate catalysis, because most of these processes are driven by extracellular or intracellular enzymes. Enzyme activity determines the biodegradation of organic compounds passing through the soil profile and it is in many cases a limiting factor in NOM decomposition. Easily available organic compounds are rapidly assimilated and make up only a small portion in the infiltrated water. Macromolecules are decomposed into smaller compounds suitable for microbial uptake by enzymes produced by heterotrophic bacteria. (Miettinen et al. 1996; Kolehmainen et al. 2009a; Schütz et al. 2010)

Free enzymes are short-lived because they can be rapidly denatured, degraded or inhibited. On the other hand enzymes can be bound and accumulated to soil particles and humic colloids, and they can persist also under harsh conditions. (Schütz et al. 2010) Depolymerisation of polysaccharides, dephosphorylation of organic molecules, and hydrolysis of the peptide bonds in proteins is carried out by glucosidases, phosphatases and aminopeptidases, respectively, which are the most important extracellular enzymes in humic surface waters (Miettinen et al. 1996; Kolehmainen et al. 2009a).

The aquifer bacterial communities are dominated by diverse heterotrophic Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes. Several mostly uncultivated lineages have also been frequently detected (for example Acidobacteria, Chloroflexi, Verrucomicrobia and Nitrospirae), as well as phyla for which cultured representatives are totally unknown (for example the OP, WS, and TM7 candidate divisions). All of the detected lineages occur also in other environments but there are many indications that the subsurface microbial communities are distinct from communities in soil and surface waters. The differences are apparent by the specific phylogenetic composition of groundwater microbial communities and by their special physiological capabilities. (Giebler and Lueders 2009)

Numerous abiotic and biotic parameters may directly or indirectly control microbial diversity in aquifers. The diversity is also affected by spatial heterogeneity, temporal variability, and disturbances such as pollution (Giebler and Lueders 2009). The geological media has been shown to affect bacterial community composition in a pristine aquifer, but how the microbial community structure and diversity at the particle scale relates to that at the aquifer scale, is not yet determined (Boyd et al. 2007). Rousk et al. (2011) demonstrated that soil microbial community composition radically differed along a pH gradient, but this did not affect the mineralization of low-molecular-weight carbon source. It has also been hypothesized that microbial life strategy may play a role in NOM biodegradation and shifts in microbial communities. Slow-growing oligotroph K-strategists have a longer life cycle and better withstand nutrient depletion, having ability to degrade refractory NOM better compared to the fast growing r-strategists characterized by a short life cycle and specialisation to use the labile, easily metabolizable organic substrates. (Kolehmainen 2008; Schütz et al. 2010) However, the microbial community composition changes along with changes in environmental conditions and thus the influence of populations on NOM biodegradation is difficult to detect (Kolehmainen 2008).

2.2.4. NOM biodegradation potential in different zones in MAR

It is widely demonstrated that most of the organic matter is removed in the upper sediments and in the beginning of the infiltration. A few studies investigated more detailed the role and structure of water-sediment environment and the upper sediments at Lake Tegel bank filtration site in Berlin, Germany. The upper sediment was shown to be a highly active biological zone of about 10 cm depth built up by interstices algae, bacteria, produced extracellular polymeric substances and meiofauna. The upper part of the boundary layer served as a filter with a high retention capacity for fine particles with sizes down to 2,44 μm as well as for natural particulate organic matter (POM). No significant particle transport to deeper sediments was observed, which indicated that easily degradable organic matter was rapidly removed due to biodegradation, decomposition and grazing. Complete biological clogging was not observed in the bank filtration site. Reasons for this were assumed to be high carbon turnover, wave action

probably reopening the interstices and feeding activity by meiofauna and other microorganisms. The clogging in bank filtration is different from the compact, surficial clogging layer regularly observed during slow sand filtration. (Gunkel et al. 2009; Hoffmann and Gunkel 2011a)

At the same bank filtration site, high DOC mineralization rates were observed in the upper sediment layer indicating very fast turnover reactions and consumption of oxygen. About 13-20% of DOC was removed in the upper 1-26 cm, while during the entire sediment passage DOC was removed by 20-50%. In summer, the turnover of the infiltrating DOC contributed 25% to the entire organic carbon mineralization in the uppermost 10 cm of sediment while in winter DOC via infiltration contributed nearly 50% to mineralization. It has been proposed that aerobic oxidation of organic matter in sediments primarily depends on its biodegradability instead of its quantity and thus it was proposed that at the bank filtration site, fresh POM may be a better nutritional source compared to the infiltrating DOC, because it releases DOC and at the same time offers a colonization surface to microbes as well as biofilms. (Hoffmann and Gunkel 2011b)

Fresh organic matter, regardless of redox conditions, is easier to become mineralized than old, partially degraded or adsorbed organic matter, which is more efficiently mineralized under oxic conditions (Bastviken et al. 2003). On the other hand, also the quantity of NOM has been reported to affect biodegradation and microbial communities. (Kolehmainen 2008; Frette et al. 2009) In addition to the quality and quantity of NOM, the microbial community composition affects the NOM biodegradation potential in aquifers and soils, but the roles of different bacteria and microbial communities for biodegradation has not yet been disclosed (Långmark et al. 2004; Giebler and Lueders 2009).

Although it is widely demonstrated that major NOM removal occurs in the beginning of the infiltration process, contradictory results about NOM biodegradation also exist. It has been noticed, that significant NOM removal can also occur in vadose zone and saturated zone and that the potential of microorganisms for biodegradation in these zones should not be underestimated. Helmisaari et al. (2006) demonstrated that TOC reduction was mainly restricted to the groundwater zone at five Finnish AGR sites. At one of the sites, Kortelainen and Karhu (2006) demonstrated that 44% of DOC decrease was due to biodegradation in the groundwater zone. Other studies have also demonstrated high DOC removal rates in vadose zone at AGR sites by both biodegradation and adsorption (Kortelainen and Karhu 2006).The site-specific conditions cause differences in NOM biodegradation potential and also sampling techniques play an important role in different studies. (Kolehmainen 2008; Schütz et al. 2010)

2.2.5. The role of temperature and redox conditions in NOM degradation

Water temperature has an important role in the performance of MAR processes. It affects bacterial activity, production, and abundance, and that way the biodegradation of organic matter. It also affects the physiochemical characteristics of the environment. (Groß-Wittke et al. 2010; Maeng et al. 2010) Low temperatures cause adaptations in cellular composition and physiology of microorganisms, and reduce microbial activity. This may increase the substrate requirements, and it has been concluded that small temperature changes can significantly affect the affinity of microorganisms for the substrates. (Kolehmainen 2008) Increased microbial activity and accelerated chemical and enzymatic reactions caused by the increased temperature may have a positive influence on biotransformation of organic matter. (Kolehmainen 2008; Groß-Wittke et al. 2010; Hoppe-Jones et al. 2010)

Increased temperatures may lead to increased oxygen consumption which, combined with reduced oxygen solubility, can significantly decrease the redox potential in sediments. If oxygen depletion occurs, alternative electron acceptors are used step by step. Various studies have reported the temperature dependency of redox processes, such as denitrification. The purification process in MAR is redox-dependent biodegradation of organic material, and turnover rates are highly dependent on local redox conditions as well as on temperature. (Groß-Wittke et al. 2010) Furthermore, some organic compounds are sensitive to different hydrogeochemical environments and, thus, redox conditions have an important role by promoting or retarding the removal of these compounds (Maeng et al. 2010).

It has often been reported that in MAR processes the anaerobic microbial community is less effective in DOC removal, and metabolic processes are slower compared to aerobic microbial communities (Groß-Wittke et al. 2010). Grünheid et al. (2005) reported nearly identical residual DOC concentrations at bank filtration and artificial recharge sites with different redox conditions. At an oxic AGR site the removal was fast but the residual DOC-concentration remained stable during further infiltration, whereas under anoxic conditions at the bank filtration site DOC continued to decrease still after 2,8 months. Bastviken et al. (2003) reported similar oxic and anoxic mineralization rates in lake sediment which consisted to a greater extent of labile organic matter, but greater oxic mineralization rates in sediment containing more refractory organic matter.

A commonly supported view is that bacterial growth rates and organic matter mineralization rates under oxic conditions are faster than anoxic rates. However, many studies have found little or no difference between anoxic and oxic rates. (Bastviken et al. 2003) Anaerobic biodegradation processes have proved to be efficient for organic matter that is easily accessible to degradation without the participation of oxygen, such as polysaccharides, proteins, fats and nucleic acids. These compounds are hydrolyzed

through specific extracellular enzymes, and the oligo- and monomers can be degraded inside the cell. The specific activities of such enzymes in anaerobic cultures are in the same range as those of aerobic bacteria, and thus the transformation rates per unit biomass should be equivalent. However, anaerobic bacteria obtain far less energy from substrate turnover than aerobic bacteria, and therefore anaerobic bacteria can produce less biomass per substrate molecule than aerobes can. In the presence of molecular oxygen, organic recalcitrant compounds are attacked biochemically through oxygenase reactions, which introduce molecular oxygen into the respective molecule. Oxygenase reactions cannot be employed in the absence of oxygen, and therefore compounds that require oxygenases for aerobic breakdown might resist degradation under anoxic conditions. (Schink 2004) Similar oxic and anoxic degradation rates can be expected for fresh, labile organic matter, whereas oxic degradation is more rapid and extensive for partially degraded or adsorbed old, recalcitrant organic matter. Bacterial biomass production may be strictly dependent on the electron acceptor used whereas organic matter degradation is not as tight depending on redox conditions, but is rather regulated by the enzymatic capacity and the organic matter quality. (Bastviken et al. 2003)

2.2.6. The role of sorption in organic matter removal

In addition to biodegradation, adsorption is characterized as another major removal mechanism of organic matter during MAR. In adsorption, molecules and ions are bonded and adhered onto particle surfaces by a combination of physical and chemical processes. Adsorption is a non-sustainable removal mechanisms because in desorption molecules are released from the particle surfaces and eventually a breakthrough of organic matter could result in MAR systems. In addition, adsorption can cause clogging, which decreases the permeability and results in reduced infiltration rates. (Bouwer 2002; Fox et al. 2005; Kolehmainen 2008) However, sorption can lead to increased residence time of organic matter and therefore it may enhance biodegradation of NOM (Grützmacher and Reuleaux 2011).

The characteristics of sorbent, sorbate, and water influence sorption. Aromatic, large molecular sized, recalcitrant and hydrophobic organic matter has been reported to adsorb more readily than hydrophilic and labile compounds. Several studies have demonstrated that the mobility of NOM increases with decreasing molecular weight and hydrophobicity (Tufenkji et al. 2002). The clay content in soils affects the sorption efficiency of humic substances and the water pH affects the surface charge of some clays and this way also sorption. (Kolehmainen 2008)

Several studies have determined adsorption of organic matter in reclaimed water in column and field site studies. The results have demonstrated that less than 20% of organic carbon adsorbs to soil particles. Accumulation of organic carbon has been reported at the surface sediments, presumably due to biological activity, but no evidence of organic carbon accumulation was observed in deeper soil depths. However, a

moderate removal of organic matter in a groundwater zone due to adsorption has also been reported in an AGR site by Kortelainen and Karhu (2006). A study with different soils showed similar organic carbon accumulation for different soils and operational conditions. The selected soils represented a wide range of soil properties that might be used during MAR. (Fox et al. 2005)

2.3. Detrimental components in groundwater and raw water sources

Surface water from streams, lakes and canals, storm water runoff, and treated drinking water can be used for aquifer recharge, but they all can be exposed to permanent or sudden pollution. The development of analytical procedures and instruments has enabled the quantification of environmental contamination at very low concentrations and detection of a large number of organic micropollutants in the aquatic environment and drinking water (Maeng et al. 2011a). Many aquifers have been contaminated by pollutants from industrial, municipal, and agricultural activities, and the remediation of a polluted aquifer is difficult and expensive. The occurrence of micropollutants in source waters, aquifers, and even in drinking water has awoken concern of pollution of aquifers used for managed aquifer treatment and effects of the organic micropollutants for human health. These pollutants include pesticides, pharmaceuticals and their metabolites, household chemicals, personal care products, disinfection by-products, insecticides, endocrine disruptors, industrial chemicals, metals, and pathogens. (Díaz-Cruz and Barceló 2008; Laws et al. 2011)

The fate of especially pharmaceutically active compounds (PhACs), endorcine disrupting compounds (EDCs) and personal care products (PCPs) in managed aquifer treatment process have gained a lot attention in the last 15 years (Hoppe-Jones et al. 2010). These compounds exist usually at trace concentrations in the environment, but their effects on human health and the environment is not fully understood. However, toxicological surveys have found that low concentrations of these compounds can cause unfavourable effects on aquatic life. (Onesios et al. 2009) Various combinations of PhACs, EDCs and PCPs might have different and synergistic impacts on public health and aquatic life (Maeng et al. 2011a), their chemical persistence is not fully known (Onesios et al. 2009), and, in addition, antimicrobial residues have raised the issue of drug-resistant bacteria formation (Heberer et al. 2008).

Point source pollution of waters used for infiltration can be controlled by accomplishing legislation and directives but the risk of water contamination remains through accidents causing uncontrolled chemical spills. Agriculture and animal farms cause the most important non-point source pollution which is more difficult to prevent. Used source water and groundwater can become contaminated from pesticides, herbicides and fertilizers spread on fields, and from antibiotics and hormones or their metabolites in

urine and faeces. Groundwater contamination occurs directly by percolation and indirectly via surface water contamination. (Díaz-Cruz and Barceló 2008)

In areas with dense population, water affected by municipal and industrial sewage is often used indirectly for potable use via managed aquifer recharge (Heberer et al. 2008). These effluents are an important source of contamination because in conventional water treatment processes many compounds have been noticed to be poorly or only partly removed. Sewage effluents contain for instance flame retardants, plasticizers, pharmaceuticals, and personal care products, some of them known to be endocrine disrupters. Reclaimed wastewater must be extensively treated in regions where it affects drinking water supply. Also water originating from industrial areas, airports and marinas can be highly contaminated. Storm or rain water runoff can contain pesticides, herbicides, polar compounds photochemically formed in the atmosphere, and a wide variety of chemicals washed up by the rain water. (Díaz-Cruz and Barceló 2008)

Aquifers have a limited and not totally reliable capacity to attenuate and purify the filtrated water. Groundwater environments are characterized by long residence times, low temperatures, decreased microbial population, and possibly low degrees of dilution which all favour the stability of organic contaminants. (Díaz-Cruz and Barceló 2008) During soil passage, organic compounds are removed via various mechanisms from which biodegradation is the most important and desirable. This is because the biodegradation process can mineralize organic compounds into inorganic compounds, thus being a sustainable removal mechanism. Sorption, for its part, also has an impact on the bioavailability of contaminants. (Maeng et al. 2011a) If the adsorbed contaminants do not break down, they will accumulate in the aquifer and they may have long-term impacts. Chemicals with low biodegradation and sorption properties will move with the water through the aquifer being poorly reduced. (Díaz-Cruz and Barceló 2008)

Managed aquifer treatment has been shown to be capable to significantly remove turbidity, natural organic matter, pesticides, pharmaceuticals, salinity, and taste- and odor-causing compounds. With sufficient filtration times and flow path lengths, MAR has been shown to provide a barrier to microorganisms and significantly reduce the presence of Giardia and Cryptosporidium. (Dash et al. 2010) In the following chapters the removal processes of common pollutants and undesired compounds during MAR are represented.

2.4. Iron and manganese removal in MAR

Iron (Fe) and manganese (Mn) are both commonly present in groundwater. In drinking water Fe and Mn cause colour and taste, and the guidelines for the concentrations in drinking water are rather aesthetical. Fe is not harmful for human health but in higher concentrations Mn has been hypothesized to impair children's intelligence (Farnsworth and Hering 2011) or to be toxic. The natural concentrations of Fe and Mn in groundwater and in aquifers is affected by the geological formation of the aquifer, Fe and Mn concentrations in soil, shallowness of the groundwater level, and redox conditions. Redox conditions and biochemical processes both influence the retention and precipitation of Fe and Mn in an aquifer. (Karttunen and Tuhkanen 2003)

Although Fe and Mn are relative simply removed after MAR, for example by aeration and sand filtration, they can cause well screen clogging and indicate conditions where Fe and Mn oxide associated trace metals are being released along the flow path. Mn and Fe oxides are major sinks for trace metals and they are released when Mn and Fe oxides are reduced. These risks and the need of post-extraction can be avoided by *in situ* immobilization. (Farnsworth and Hering 2011)

In the environment iron has two oxidation states (+II and +III) and manganese three (+II, +III, and +IV). Fe(III), Mn(III) and Mn(IV) are found in oxides and oxyhydroxides which are largely insoluble but liable to dissolution under reducing conditions. At low to neutral pH Fe(II) and Mn(II) are soluble. Depending on the carbonate concentrations they can precipitate as siderite ($FeCO_3$) and rhodochrosite ($MnCO_3$) or, at very alkaline pH values, as $Fe(OH)_2$ and $Mn(OH)_2$. Reductive dissolution of Fe and Mn oxides and immobilization of dissolved Fe and Mn in water depend on the variation of the redox state along the flow path. (Farnsworth and Hering 2011) Changing redox states and reducing conditions are commonly observed at different bank filtration and artificial recharge sites (Massmann et al. 2006).

In MAR processes oxygen and often nitrate can be consumed in groundwater due to microbial activity. This leads to reductive dissolution of Fe and Mn oxides, and to Fe and Mn release to sediment porewater. When oxic conditions in the aquifer are returned by diffusive flux from the overlying unsaturated zone, vertical infiltration of oxic rainwater, or by gas entrapment due to water table oscillations, dissolved Mn and Fe may precipitate as oxides. Reductive dissolution of Fe and Mn oxides are mostly microbially mediated, as well as some oxidative precipitation of Fe(II) and Mn(II). Microbial Mn oxidation is faster compared to abiotic Mn oxidation which is slow below pH 9. At near neutral pH, rapid abiotic Fe(II) oxidation reaction is dominant. (Farnsworth and Hering 2011)

Disequilibrium between infiltrating water and native groundwater causes sorption and ion exchange, and can lead to mineral dissolution or precipitation during the MAR process. Carbonate minerals are most liable to dissolution, and although carbonates are not major sinks for trace elements, they can control dissolved Fe, Mn, and other trace cation concentrations in aquifers with sufficiently high alkalinity. Due to water disequilibrium, surface-associated ions of the aquifer material may desorb, while ions from the bank filtrate may adsorb on sediment surfaces. Sorption is the main retardation process for dissolved components and it has an important role in iron in-situ removal. Both surface area of the sorbent and the affinity of the dissolved species for the sorbent surface sites affect sorption. In *in-situ* iron removal, aerated groundwater is injected into the aquifer where it precipitates adsorbed Fe(II) to Fe oxides, offering new absorption surface for dissolved iron. In long-term aquifer treatment, sorption can lead to saturation of the aquifer sediment surfaces and in the end to a breakthrough of dissolved components in the production well. Dilution with native groundwater and dispersion can significantly damp any sorption front moving through an aquifer but on the other hand a change in the composition of infiltrated water can easily cause desorption leading to a conclusion that sorption is not alone enough to tong-term sequestration of Fe, Mn or other trace elements. (Farnsworth and Hering 2011)

2.5. Cyanotoxin removal in MAR

Different genera of cyanobacteria, earlier called blue-green algae, can produce lethal toxins being classified to hepatotoxins, neurotoxins, and lipopolysaccharides endotoxins, according to their chemical structure. Hepatotoxins, also called microcystins, are considered the most common among cyanotoxins, and at least 76 structural variants have been identified in cyanobacterial blooms. Microcystin-LR and microcystin-YR are the most toxic variants (Grützmacher et al. 2002), microcystin-LR being the most common variant causing liver hemorrhage within few hours and showing tumor promotion activity through protein phosphatase inhibition. Cyanotoxins pose a hazard for animals and people particularly through oral ingestion, thus, a provisional guideline value of 1,0 μg/l for microcystin-LR in drinking water has been set by the World Health Organization (WHO 1998). (Eleuterio and Batista 2010)

Cyanobacterial blooms can be observed in surface waters worldwide. Elevated nutrient concentrations, particularly phosphorus and nitrogen, and low turbulence can cause accumulation of cyanobacterial blooms (Eleuterio and Batista 2010). In healthy cyanobacterial populations usually more than 90% of cyanotoxins are contained within the cells, but when a population ages, dissolved microcystins can be observed (Grützmacher et al. 2002). Cyanobacterial blooms and cyanotoxins in high concentrations in source waters and in shoreline accumulations pose a risk for artificial recharge and bank filtration processes, and, thus, the elimination of the microcystins has to be ensured when microcystin contaminated water is used to produce drinking water.

Some studies have shown that microcystins detected in surface water can influence groundwater leading to the conclusion that breakthrough of microcystins is possible in bank filtration and artificial recharge systems. (Grützmacher et al. 2010)

Elimination of microcystins has been widely studied in natural waters and soil matrixes. It has been shown that physical filtration of cell-bound microcystins is the main removal method in full scale slow sand filtration process. Adsorption of microcystin to sandy material has been demonstrated to be low and sorption processes reversible, and it has been concluded that in natural systems abiotic degradation has minor importance in microcystin elimination compared to biodegradation. (Grützmacher et al. 2002; Grützmacher et al. 2010)

Microcystins can be used as a carbon source by heterotrophic bacteria and the biodegradation of microcystins has been showed to be effective leading to their total degradation (Eleuterio and Batista 2010). In natural surface waters cyanotoxins can persist for long periods before they degrade. It is supposed that the conditioning and induction of an endemic microbial population, as well as environmental factors like temperature, pH, and predation by protozoa, affect the length of acclimation period, after which degradation is often fast completed. (Bournea et al. 2006) Also in lab-scale biofilters lag-times of a few days have been reported after which rapid degradation of microcystins have occurred in optimal conditions. It is assumed that the majority of microcystin degradation is most likely to take place in the contact layer of water and filter matrix as most biological activity in biological filters occur in this layer. (Grützmacher et al. 2002)

Grützmacher et al. (2010) investigated elimination of four microcystin variants in batch and column experiments. Adsorption was confirmed to be an unsustainable and minor elimination method, but offering a longer time for microcystin degradation. Microcystin variant, geochemistry and grain size distribution of sediment, and organic material were discovered to affect adsorption. Biodegradation was identified as the dominant process eliminating 84-100% of the initial microcystin concentration. Under near-natural aerobic conditions, high degradation rates could be achieved, but some different experimental conditions like water characteristics influenced them. The results suggested that biodegradation may occur without the establishment of a microbial population specialized on microcystin degradation, and that attached microbial populations seem to have more important role in biodegradation compared to unattached populations. Lag phases appeared to occur only when disadvantageous conditions for microbial activity existed. Anoxic degradation rates were generally significantly lower and varying for different microcystin variants. (Grützmacher et al. 2010) Successful biodegradation has also been observed in other lab-scale and full-scale biofilters without microcystin degrading bacterial inoculation leading to suggestion that

microcystin-degrading bacteria may be prevalent in the environment (Grützmacher et al. 2002; Bournea et al. 2006; Eleuterio and Batista 2010).

Li et al. (2011) studied bacterial community compositions during accumulation and breakdown of *Microcystis sp.* blooms with in-situ mesocosm column studies. The results showed that the accumulation, breakdown and different levels of Microcystis blooms all affected strongly both free living and particle attached bacterial community composition. The changes were detected as disappearance and appearance of different bacterial populations and as changes in dominant populations. (Li et al. 2011)

Eleutero et al. (2010) noticed in their study that additional biodegradable carbon source significantly repressed the biodegradation of microcystins. In the experiments most of the easily biodegradable TOC was rapidly degraded while microcystins degradation was low. Microcystin degradation was faster in the absence of biodegradable TOC. Likewise, Grützmacher et al. (2010) recognized residual microcystin concentrations only in the presence of additional dissolved organic matter. Degradation competition between easily biodegradable natural organic matter and cyanobacterial toxins could lead to a preferred degradation of DOM as a primary substrate for toxin-degrading bacteria (Grützmacher et al. 2010). Thus, ability of biofilters to degrade microcystins can depend on the biodegradable NOM and toxin concentrations in the influent water. (Eleuterio and Batista 2010)

Low water temperatures have also been assumed to hinder complete microcystin elimination by biodegradation (Grützmacher et al. 2002). Holst et al. (2003) showed that anaerobic microcystin degradation can be significantly stimulated by the addition of nitrate, and it was assumed that microcystin degradation is coupled to dissimilative nitrate reduction (denitrification). Since aquifers and sediments can be anoxic in MAR, nitrate respiration may be an important process in anoxic microcystin elimination. In the future, the composition and density of the microbial populations as well as the role of electron acceptors for the microorganisms in microcystin degradation should be studied. (Grützmacher et al. 2010)

2.6. Pathogen removal in MAR

Health risks from microbial pathogens cause concern especially when non-potable water sources, for instance treated effluents or effluent influenced waters are used as a water source in managed aquifer recharge. The possibly high abundance of pathogens in the source water makes their removal by retention or inactivation in the aquifer a key parameter in regions with high risks of pathogens. (Anders and Chrysikopoulos 2005; Metge et al. 2010; Toze et al. 2010) The removal of specific enteric pathogens and indicator microorganisms due to decay in the aquifer has been recognized for some time, and pathogen removal by MAR has been demonstrated in several studies. Thus,

MAR is being used as a treatment barrier to remove pathogens from the recharged water, and understanding the most influential factors and dominant removal processes in aquifers is fundamental for the management and design of artificial recharge systems. (Gordon and Toze 2003; Toze et al. 2010)

The most common microbial pathogens found in waters are enteric in origin and can include viruses, bacteria and protozoa. The risk of water-born infection depends on factors like pathogen number and dispersion in water, the infective dose required, and the susceptibility of an exposed population. Enteric pathogens enter water through faeces of infected hosts, through contamination with sewage effluent, or from run-off from soil and other land surfaces. (Toze 2006)

More than a third of waterborne illnesses are attributed to enteric viruses which may remain infectious for many months under suitable conditions (Långmark 2004). In water viruses are present as inactive particles because they lack the ability to self-replicate. Most enteric viruses have a narrow host range meaning that most viruses of interest in recycled water only infect humans. In wastewaters, a wide range of bacteria have been detected and they are the most common microbial pathogens found in recycled waters. Since bacterial pathogens are capable of self-replication, they are theoretically capable of replicating in the environment. The transmission of bacteria, as well as other enteric pathogens, happens via contaminated water or food, by person to person contact or by wild and domestic animals. Single-celled or colonial protozoa are also found widely in water habitats. Most of them are non-parasitic, but some species like *Giardia* and *Cryptosporidium* can cause infection (Långmark 2004). Protozoa persist outside of a host as dormant stages known as cysts or oocysts which can further infect new hosts. (Toze 2006)

Biological filtration is an effective method for the reduction of microorganisms present in source water and it has been recognized that microbial pathogens loose viability in groundwater (Gordon and Toze 2003; Långmark 2004). Differences between the decay of pathogens in different aquifer paths have been observed, and the retention and elimination of pathogens is influenced by several factors (Stevik et al. 2004; Toze et al. 2010). Most of the large microorganisms can be efficiently removed from source water but certain amount of particles and particle-attached microorganisms can penetrate filtration processes. Thus, biological filters do not represent an ultimate barrier against microorganisms. The mechanisms for the pathogen immobilization are a combination of physical straining, adsorption, and abiotic and biotic factors. The factors affecting the elimination of pathogens include moisture content, pH, temperature, dissolved oxygen, organic matter, bacterial species, predation, and the presence of indigenous micro-organisms. (Gordon and Toze 2003; Långmark 2004; Stevik et al. 2004)

In the retention of pathogens, the grain size of porous media is an important factor influencing straining. Silt, clay, and fine sand have pore sizes within the range of most bacterial cells and thus straining can be a mechanism limiting the movement of pathogens. Also the size and shape of pathogens influence their transport. (Stevik et al. 2004) Sorption becomes the dominant mechanism for retention when the pores are larger than the pathogens and it has been observed to be the major retention process for viruses (Schijven et al. 1999; Anders and Chrysikopoulos 2005).

The degree of pathogen sorption during subsurface transport is affected by several factors including sediment surface charges and characteristics, viral and bacterial surface properties, pH, the presence of organic matter, and extracellular polymer production. On one hand, organic matter attached to the media may increase the cation exchange capacity, surface area, and number of adsorption sites for bacterial adsorption, but on the other hand, organic matter in water phase can reduce bacterial sorption to soil sediments by offering and alternative adsorption site. (Stevik et al. 2004) Organic matter and particles can function as transport vehicles for attached pathogens and provide increased protection from environmental, chemical and biological stresses (Långmark 2004).

Few studies (e.g. Schijven et al. 1999; Hoffmann and Gunkel 2011a) have indicated that most of the pathogen removal in MAR occurs in upper, fine sediments and in the beginning of infiltration path. Metge et al. (2010) discovered that also the deeper, coarser sediments can exhibit a high efficiency for removal of oocyst-sized colloids. This removal was a function of the iron and aluminum oxide content on the grain surfaces and it was suggested to be sorptive filtration rather than physical straining. DOC addition in water decreased sorption significantly, up to 35,7%, pointing out the role of organic matter in pathogen removal.

In biological filtration, elimination of pathogens is controlled by abiotic factors like moisture content, pH, temperature, dissolved oxygen, and organic matter content. Biotic factors affecting pathogen elimination include bacterial species and other microorganisms. Increasing temperature has been shown to decrease bacterial and viral survival. (Gordon and Toze 2003; Stevik et al. 2004) The exact mechanism of inactivation by temperature is uncertain, but it is assumed that increased temperature could cause thermal degradation of the viral capsid, and control the activity of other inactivation mechanisms, such as the metabolic activity of indigenous groundwater microorganisms. Thus, it is assumed that temperature has mainly an indirect influence of bacteria and viruses. (Gordon and Toze 2003)

Likewise, the presence of oxygen may have an indirect influence on pathogen decay. Virus inactivation is reported to increase by increased dissolved oxygen. Oxygen may directly inactivate viruses by oxidation of the viral capsid, or similar to the role of

temperature, indirectly affect pathogen inactivation by increasing the activity of indigenous microorganisms. (Gordon and Toze 2003) Castro et al. (2008) investigated the effect of variations in the dissolved oxygen concentration on pathogenic bacteria mobility, and found that influence of oxygen on bacterial transport and deposition is strongly dependent on the species. Variation of oxygen concentrations during bacterial growth and acclimation significantly affected cell surface charge, electrophoretic mobility, and cell size and shape.

Organic carbon has been implicated to influence the decay of pathogens in groundwater probably due to increase in the activity of the indigenous microorganisms. Alternatively, as earlier discussed, it has also been reported that survival of pathogens may be lengthened in soils containing organic matter, probably due to the protective influence of organic matter. (Gordon and Toze 2003; Stevik et al. 2004)

Different pathogens can have various survival times and especially within pathogenic bacteria the survival time has been reported to differ significantly. Reasons for this might be their ability to compete for nutrients with indigenous microorganisms, or they may be less sensitive to antibiotics produced by soil bacteria. Different bacterial species show also variation in their ability to withstand low and high water contents. Longest survival times have been found in moist soil and saturated conditions, whereas dry conditions can significantly increase bacterial reduction rates in soil. Bacteria that can produce spores can survive longer under unfavorable conditions compared to those that cannot. (Stevik et al. 2004) In addition to aquifer residence time and pathogen decay rates, initial pathogen numbers in water have also been shown to be an important variable in MAR microbial risk assessment (Toze et al. 2010).

The activity of the indigenous groundwater microorganisms is known to be important in pathogen decay. The study of Gordon and Toze (2003) showed it to have a major influence on the inactivation of enteroviruses and indicator micro-organisms. The site specific aquifer characteristics influence the pathogen decay ability of the native groundwater microorganisms. (Gordon and Toze 2003; Toze et al. 2010) In the environment pathogens are subject to predation, and exposed to inhibitory substances, bacterial phages, protozoa and nematodes. Some of these factors and competition for nutrients with indigenous bacteria may also be reasons for reduction of enteric bacteria in soil. (Stevik et al. 2004) Even though there is a reasonable amount of information on the decay of indicator microorganisms (for example *Escherichia coli*) and the more easily culturable enterovirus groups, there is still need for more information on decay rates of many enteric pathogens such as the less easily studied viruses and protozoa in groundwater (Toze et al. 2010).

2.7. Pharmaceuticals and trace organic contaminants

2.7.1. Removal of pharmaceuticals and trace organic contaminants in MAR processes

Water utilities using MAR are commonly located downstream from populated regions. Thus, the source water used for bank filtration or artificial recharge is often affected by wastewater effluents and agricultural runoffs and can contain pharmaceutically active compounds, endocrine disrupting compounds, personal care products, industrial chemicals and pesticides. (Maeng et al. 2011b) Although these compounds are often found in very low concentrations in water and harmful human health effects are unlikely, it is desirable to minimize the exposure to these contaminants (Rauch-Williams et al. 2010). Investigation of the fate and attenuation of pharmaceutically active compounds and other trace organic chemicals is necessary in order to determine the requirements for pre- or post-treatment in MAR systems and to design new water treatment plants with bank filtration or artificial recharge. (Maeng et al. 2010; Maeng et al. 2011b) Recently studied PhACs commonly occurring in surface waters can be grouped into eight categories according to their therapeutic uses (Maeng et al. 2011b).

Antibiotics

The most important pharmaceutical group is antibiotics which can originate from human and veterinary medicine, plant agriculture and aquaculture. Concerns about the effects of antibiotics on aquatic environment exist because of the possible existence and growth of antibiotic resistant microorganisms. It has also been shown that a long exposure to antibiotics can significantly reduce biological processes such as nitrification. (Maeng et al. 2011b) Several studies have demonstrated that among all antibiotic residues sulphonamide sulfamethoxazoles are the most relevant as potential contaminants for groundwater (Heberer et al. 2008).

Grünheid et al. (2005) discovered better removal of sulfamethoxazole in a BF site characterized by varying oxic and anoxic conditions and longer retention time compared to an AGR site with oxic conditions and shorter retention time. Likewise, Laws et al. (2011) noticed low sulfamethoxazole attenuation of less than 10% after infiltration through the vadose zone but increased attenuation of 42% with increased retention time in aquifer. In a study of Heberer et al. (2008), 99% of sulphonamide sulfamethoxazole were eliminated under anoxic infiltration conditions, while the attenuation was 52% under seasonally oxic conditions. Anoxic conditions were concluded to be advantageous for the degradation of sulfamethoxazole. Other investigated antibiotics have been noticed to be removed effectively by MAR processes in several BF and AGR sites with sufficient retention times and travel distances, regardless of the influence of redox-potentials (Heberer et al. 2008; Maeng et al. 2011b).

Non-steroidal anti-inflammatory drugs and analgesics

High consumption of non-steroidal anti-inflammatory drugs (NSAIDs), also known as pain killers, has lead to detection of the compounds in wastewaters and aquatic environments. Based on high octanole water partition coefficients, sorption has been suggested to be the main removal mechanism for diclofenac, ibuprofen, naproxen and indomethacin during soil passage. However, several studies have also shown biodegradation potential for some NSAIDs in laboratory-scale. (Maeng et al. 2011b) Few studies have reported moderate or low attenuation for diclofenac, ibuprofen, and naproxen in the beginning of infiltration, but good removal of the compounds with increased travel times (Laws et al. 2011).

The majority of NSAIDs are acidic compounds that exist as ionic species during soil passage. Thus, ionic interactions may have more influence on the removal of acidic NSAIDs than sorption does. This affects their fate in aquifer, and makes pH an important parameter in their investigation. Redox conditions have been discovered to strongly affect the removal of phenazone-type pharmaceuticals and their metabolites. Very high removal efficiencies have been observed for most of these compounds under oxic conditions, and biodegradation by aerobic bacteria has been suggested to be the main removal mechanism. Many investigated NSAIDs have been removed at rates greater than 50% during BF and AR, and depending on the NSAID, pH, redox conditions, and travel times and distances can have an influence in their attenuation. However, good removal efficiencies despite of short residence times and short travel distances have also been reported. (Maeng et al. 2011b)

Anticonvulsants

Anticonvulsant pharmaceuticals (for instance dilantin, primidone and carbamazepine) have been reported to degrade poorly (< 10%) or not at all in field and column studies even after years of retention time. Some studies have suggested varying degrees of adsorption of carbamazepine onto soil and wastewater solids and no retardation of primidone. (Hoppe-Jones et al. 2010) Laws et al. (2011) discovered < 10% removal of the two compounds in the beginning of infiltration and increasing attenuation with increased travel time. However, the reduction was assumed to be a result of dilution with native groundwater because it has been observed that carbamazepine and primidone do not easily biodegrade or sorb well to soil particles. (Laws et al. 2011) Many studies on anticonvulsants have proven that MAR processes are not effective for the removal of these compounds. (Maeng et al. 2011b)

Antidepressants

Limited information on the removal of antidepressants in MAR processes is available due to the minor amount of studies conducted. In field studies over 90% of fluoxetine was rapidly removed after a short infiltration time (Laws et al. 2011) and in column studies the compound was almost completely removed. The removal was attributed

mainly to sorption. Removal for another antidepressant, meprobamate, was 66% in the same column study. More research is still needed to characterize the performance of MAR processes regarding the antidepressant removal. (Maeng et al. 2011b)

Beta blockers

Beta blockers exist in aquatic environments often in the ng/l to µg/l range because they are not efficiently removed during waste water treatment processes. More than 70% of atenolol, metoprolol, bisoprolol and sotalol have been observed to be removed at bank filtration site along the rivers Rhine, Elbe, and Ruhr, Germany. Artificial recharge site in California, U.S. removed more than 90% of atenolol within the first three days of infiltration (Laws et al. 2011). Beta blockers have shown to be efficiently removed during MAR processes but the main removal method (sorption or biodegradation) has not been clarified and thus more study is required to determine the removal methods during subsurface infiltration. (Maeng et al. 2011b)

Lipid regulators

In lipid regulator monitoring it is important to detect the active metabolites in MAR processes because the prodrugs are in an inactive form. One of the most commonly studied metabolite in MAR processes, clofibric acid, is often detected in the aquatic environment, wetlands and wastewater treatment plants. Many lipid regulators exist as ionic species during soil passage and thus the pH affects the removal mechanism of these compounds. MAR processes have been reported to remove lipid regulators and their metabolites at rates from over 50% to over 90% even after a short travel time in soil (Laws et al. 2011). However, the removal efficiency of different MAR sites varies, and no detailed information about the removal methods has been reported. (Maeng et al. 2011b)

X-ray contrast media

X-ray contrast agents can be measured as adsorbable organic iodine (AOI). AOI reduction in MAR processes has been shown to be redox-dependent and the dehalogenation of AOI is enhanced under anoxic conditions. (Maeng et al. 2010) Grünheid et al. (2005) demonstrated that the AOI removal efficiency at a BF site with anoxic conditions was higher, approximately 60% compared to an AGR site with only oxic conditions and 30% removal efficiency. Likewise, the removal of X-ray contrast agents iopromide, iopamidol, and iomeprol have been measured separately and the results have shown good, over 80% removal efficiencies at a bank filtration site with anoxic conditions (Grünheid et al. 2005; Laws et al. 2011; Maeng et al. 2011b). On the other hand, an 89% decrease in iopromide concentration within a short infiltration distance under oxic conditions has been demonstrated, which indicates that iopromide and AOI behave differently during aerobic soil passage. One suggestion for the different behavior is that iopromide is metabolized, but not mineralized during the aerobic soil passage. (Grünheid et al. 2005)

Steroid hormones

Endocrine disrupting compounds (steroids) are considered to be natural and anthropogenic compounds, which interfere with the endocrine system function. Steroid hormones can survive wastewater treatment processes and they are present in wastewater effluents. Steroid hormones are of special concern because they produce potentially adverse effects on human health and aquatic life even at concentration range of ng/l. These effects include mimicry of natural hormones, inhibition of the effects of other hormones, and inhibition or stimulation of the functions of the endocrine system. (Mansell et al. 2004)

Mansell et al. (2004) studied the removal mechanisms of 17β-estradiol, estriol, and testosterone, which are commonly detected in effluents. The primary removal mechanism of the compounds was adsorption. Removals between 79% and 98% were achieved on silica sand, and higher content of silt, clay and organic content yield higher removal efficiencies. Although it has been shown that hormones can desorb, the sustainability of the removal was shown by improved and rapid removal of the three compounds in the in the presence of soil microbial activity. Organic background matrix present, redox conditions, and flow conditions (saturated vs. unsaturated) did not affect the removal of compounds. (Mansell et al. 2004) Several other studies have also shown rapid and effective removal of other steroid hormones in MAR processes suggesting sorption and aerobic biodegradation to be the main removal methods. According to the studies, it has been concluded that MAR is an effective and reliable treatment for removing steroids. (Maeng et al. 2011b)

Other trace organic compounds

The attenuation of several other trace organic compounds has been investigated in a few studies. In a study by Hoppe-Jones et al. (2010) the concentrations of chlorinated flame retardants TCEP, TCPP, and TDCPP did not change significantly within a short travel time between the river and the production well. Laws et al. (2011) also observed poor attenuation of TCEP and TCPP in the beginning of an AGR process, but the removal of the compounds increased with increasing travel times. Although the chlorinated flame retardants have been found to be very persistent in subsurface systems, significant and high removal efficiencies attributed to biotransformation have also been reported after sufficient travel times (Hoppe-Jones et al. 2010; Rauch-Williams et al. 2010; Laws et al. 2011)

Grünheid et al. (2005) examined the removal of naphthalenedisulfonates which are intermediates for different industrial processes occurring in domestic sewage. The 1,7-isomer and the 2,7-isomer are known to be partly degradable, while the 1,5-isomer is very stable. This was also shown at BF and AGR field sites where the low reduction in concentration of the 1,5-isomer was assumed to be due to some degradation and dilution with deeper groundwater. The 1,7- and the 2,7-isomers were shown to degrade better

under aerobic conditions. In a study by Laws et al. (2011) DEET (pesticide) was poorly removed in the beginning of the AGR process, but an 84% removal was observed after longer travel time.

2.7.2. Factors influencing the removal of PhACs and trace organic compounds

Biodegradation is the desirable, sustainable removal method for PhACs in MAR processes. The characteristics of the PhACs, the specific biomass, microbial activity, and microbes present in the soil all affect the biodegradation of the compounds. The biodegradability of a compound is significantly influenced by its physicochemical properties and structural properties like branching, aliphatic functional groups, aromatic functional groups, aliphatic amines, halophenols, polycyclic aromatics, and triazines. Other important factors influencing the biodegradation potential in aquifer are the local biomass conditions including specific microorganisms present, microbial activity, available carbon source, and redox and temperature conditions. (Maeng et al. 2011b)

The organic carbon content of soils and sediments affects the sorption of non-polar organic micropollutants. The organic carbon partition coefficient, K_{oc}, is often used to evaluate the sorption and distribution behavior of these compounds but it is not proper way to estimate the behavior of compounds that contain charged sites and that exist as ionic compounds in the aquatic environment, such as acidic pharmaceuticals. For these compounds it is important to know the acid dissociation constant (pK_a), which controls the degree of ionization, and the pH to estimate the fate of the acidic micropollutants. (Maeng et al. 2011b)

Redox conditions and temperature

From many factors that affect pharmaceutical and organic micropollutant removal, redox conditions have been identified to be one of the most important. Oxidation-reduction potential values above +200 mV correspond to aerobic or oxic conditions where microorganisms use oxygen as a terminal electron acceptor during respiration. Anoxic conditions are present between -200 and +200 mV when NO_3^-, Mn^{4+} and Fe^{3+} are used as terminal electron acceptors. Values below -200 mV correspond to anaerobic conditions, when SO_4^{2-} and CO_2 are used as terminal electron acceptors. Several micropollutants are redox sensitive and, thus, redox conditions play an important role in biodegradation during MAR by promoting or retarding the removal of these compounds. (Heberer et al. 2008; Maeng et al. 2010)

In surface water detected macrolide antibiotics have been demonstrated to be readily removed in bank filtration. However, particular antibiotics were observed to be redox sensitive, and oxic or anoxic conditions were necessary to gain good removal rates. (Heberer et al. 2008) Within NSAIDs and analgesics, the phenazone type pharmaceuticals are redox-dependent and fast removed by biodegradation under oxic

conditions. Under anoxic conditions the removal is poor. The bulk X-ray contrast agents measured as AOI and anticonvulsant carbamazepine are better removed in anoxic conditions. (Massmann et al. 2006; Maeng et al. 2010) When redox-sensitive compounds exist in water used for MAR, oxic conditions followed by anoxic conditions, and varying redox conditions are effective for removing redox-sensitive organic micropollutants (Maeng et al. 2011b).

In addition to redox conditions, water temperature plays an important role in the performance of MAR processes. Temperature affects bacterial production, activity, and abundance. Redox processes have been observed to be highly dependent on the groundwater temperatures at some MAR sites. Therefore, the particular importance of the role of large temperature variations on redox-related hydrochemical changes has been acknowledged in MAR systems. (Massmann et al. 2006; Maeng et al. 2010) Massmann et al. (2006) demonstrated at an artificial recharge site, how the presence of oxygen was highly dependent on the groundwater temperatures so that at higher temperatures lower oxygen concentrations were observed. At the AGR site the temperature influenced presence of O_2 was shown to be more decisive for the phenazone-degrading bacteria than the direct temperature dependency.

Hoppe-Jones et al. (2010) observed that the average water temperature of less than 10°C in the winter resulted in reduced attenuation of trace organic chemicals. Denitrification rate was diminished, and compared to the retention in summer, at least fivefold travel times were needed to decrease the concentrations of the investigated compounds. However, the lower temperatures did not significantly affect TOC removal. Another study has also observed that the attenuation of trace organic chemicals in the subsurface was severely diminished at temperatures at 5°C as compared to 15°C. (Hoppe-Jones et al. 2010)

Organic matter and biodegradation

In case of some organic micropollutants it has been observed that the source, or characteristics, of BDOC in the infiltrated water affects the removal of the compounds in MAR (Maeng et al. 2011b). The presence of BDOC was demonstrated to enhance the removal of some trace organic contaminants in the beginning of infiltration in column experiments. It was suggested that BDOC, prevalent in form of colloidal and hydrophilic carbon, stimulated soil biomass growth and served as a co-substrate in a cometabolic transformation of the contaminants. Equal or faster removal of targeted compounds was observed in the presence of hydrophobic acids which are recalcitrant, humic-like compounds. Hydrophobic acids provided little soil biomass growth, and therefore it was assumed that they induced the development of an oligotrophic soil microbial community well capable of degrading trace organic chemicals to low residual concentrations. **(**Rauch-Williams et al. 2010)

Oligotrophic organisms are likely to be prevalent in native environments characterized by low-carbon fluxes. Therefore it is expected that these organisms reside in soil depths below the immediate infiltration zone, which in contrast is likely dominated by microbial organisms growing primarily on easily degradable organic matter. The column experiments showed that the concentration and character of effluent bulk organic carbon affect both the quantity of soil biomass growth and the composition of the microbial community, and these parameters affect the degradation efficiency of trace organic compounds during MAR. **(**Rauch-Williams et al. 2010)

Maeng et al. (2011a) studied the role of biodegradation and water source in the removal of neutral and acidic PhACs during soil passage with batch and column experiments. Certain neutral and acidic PhACs showed high removal efficiencies in different water sources with different organic matter characteristics. The removal of some acidic PhACs was assumed to depend on the amount of BDOC available, and therefore co-metabolism was expected to play an important role in their removal. The experiments showed that biodegradation represents an important mechanism for the removal of PhACs during soil passage. Low concentrations of BDOC decreased the removal efficiencies of some acidic PhACs, however, some hydrophilic neutral compounds were effectively removed under the same conditions, suggesting that these compounds may have been used as a carbon source for the growth of microorganisms. (Maeng et al. 2011a)

Other factors that have been reported to affect trace organic chemical attenuation and faith during MAR include dilution, ability of microorganisms to utilize the compounds, and the effects of contaminant association with humic substances or soil particles. It has been observed that some microbes degrade trace organic compounds more efficiently than others. For example, the removal of certain PhACs has been reported to be higher with an enriched nitrifier culture compared to the removal achieved by microorganisms from conventional activated sludge processes. The bacterial strains and consortia that grow on certain trace organic compounds have been summarized and it has been discovered that these strains consist mostly of bacteria that survive under oxic conditions. (Maeng et al. 2011b) With longer travel times in soil, dilution with native groundwater can pay significant role in the concentration reduction of certain compounds (Laws et al. 2011).

Association of some antimicrobials with humic substances has been assumed to protect the compounds from degradation processes and reduce their mobility and bioavailability to microorganisms. Lower bioavailability has been hypothesized to reduce the pressure for antibiotic resistant organisms. On the other hand, it has been shown that tightly to clay particles adsorbed antibiotic residues can still be biologically active, and therefore they may influence the selection of antibiotic resistant bacteria. (Heberer et al. 2008)

2.8. Biofilter process optimization

Microbial community control is commonly applied for example in biological wastewater treatment processes, but in biological drinking water treatment it is rarely seen at a plant scale. In denitrification filters an external carbon source is usually added to consume dissolved oxygen and to provide a food source, but in the removal of other undesired compounds microbial communities are commonly allowed to grow naturally. In microbial community control the microbial communities and processes are engineered in a way that they can efficiently remove contaminants. With a better consideration and understanding of factors affecting microbes, the correlation of phenotypic genes and the phenotypic traits, and the compounds of interest, the removal of undesired compounds, process efficiency, and operational conditions can be improved. (Zhu et al. 2010)

Culture-based microbiological analyses can be used to identify optimal environmental conditions for pure cultures isolated from the mixed microbial community, and the gained information may be used to optimize the purification process. Molecular microbiological techniques can be used to identify, quantify, locate, and track specific classes, families, genera, or species of bacteria. This way the techniques can be used to create fingerprints of bacterial communities in biological water treatment processes. Fingerprinting gives an advantage to rapidly identify the changes in microbial community when operational conditions or water quality is changing. This way it may be possible to gain better understanding about the correlations between microbial community compositions and environmental factors favouring the treatment goals. In addition to microbiological techniques, mathematical models can be powerful tools in the optimization of biological treatment systems. (Brown 2007)

Since inoculation and manipulation of biology in biofilters is mainly performed in lab-scale or pilot-scale studies, biological performance in plant scale biological water treatment is commonly enhanced by influencing the operational factors of the process. In MAR processes, these factors may include ozonation, filter media, contact time, temperature, redox conditions, nutrient additions and backwashing in conventional sand filtration. (Urfer et al. 1997; Li et al. 2010; Zhu et al. 2010; Grützmacher and Reuleaux 2011)

It has been demonstrated that the lack of nutrients required for microbial growth can prevent microbial growth in drinking water (Lehtola et al. 2002). It has also been observed that that the lack of nutrients can affect the biomass growth in MAR systems and in that way the functioning of the MAR processes (Rauch and Drewes 2005; Schütz et al. 2010). In central Europe and Northern America the limiting nutrient is generally organic carbon, especially assimilable organic carbon, whereas phosphorus has noticed

to be the limiting factor for example in Finland, Norway and Japan (Keinänen et al. 2002; Lehtola et al. 2002).

In phosphorus limiting systems, a very low increase in phosphorus concentration has been shown to significantly enhance microbial growth in water, whereas the effect of phosphate addition was different in biofilms, which showed no phosphate-dependent change in biomass. The differing results were assumed to arise from differing environments. (Keinänen et al. 2002; Lehtola et al. 2002) Phosphorus addition into two biologically active carbon reactors resulted in similar contaminant removal performances, but drastically different responses in microbial community structure were detected. Several studies have suggested that in addition to increased biomass, phosphorus addition to phosphorus-limited biological systems can enhance microbial contaminant removal, improve bioreactor performance, and affect microbial behavior at cellular and community levels. (Li et al. 2010) An organic carbon –limited system showed increasing organic carbon removal rates with increasing initial organic carbon concentrations. The study was performed in soil columns simulating MAR, and the findings indicated that microbial biomass was positively correlated to the organic carbon removal capacity. (Rauch and Drewes 2005)

In some MAR processes limitations concerning the removal of DOC occur. In these processes preozonation can be applied to improve the biodegradability of refractory organic matter and to enhance biological activity. It has been shown that ozonation increases the fraction of biodegradable NOM. Ozonation of NOM results in formation of hydroxyl, carbonyl, and carboxyl groups, increased polarity and hydrophilicity, loss of double bonds and aromaticity, and a shift in the molecular weight distribution toward low-molecular-weight compounds. (Urfer et al. 1997; Li et al. 2010)

Source water transformation during ozonation and enhancement of biodegradation by pre-ozonation were studied in lab-scale soil column experiments demonstrating two different scenarios: the first presented surface water ozonation followed by groundwater recharge, and the second presented short bank filtration of surface water followed by ozonation and groundwater recharge. Ozonation was shown to significantly increase the fraction of BDOC in water but the formation rate was lower in bank filtrate in comparison to surface water. Ozonation did not enhance selective removal of any specific molecular size of NOM, but caused formation of building blocks from humic substances, which were completely removed after soil passage. The fraction of biopolymers was not significantly affected by ozonation since biopolymers are predominantly aliphatic and not selectively attacked by ozone. (Hübner et al. 2011)

Enhanced removal of DOC was demonstrated by combining ozonation and subsurface passage. DOC removal was increased from 22% without ozonation to 40-45% with preozonation followed by groundwater recharge. With a preceding bank filtration DOC

removal was improved to 50% overall removal which was considered to have rather small relevance to drinking water treatment. The desired DOC reduction to a level of 3,5-4,0 mg/l in column effluents was reached after six days of travel time whereas at BF and AGR sites similar removal rates were observed within 3-6 months of retention. Further DOC reduction after longer retention time and biological stability of the treated water after ozonation and artificial recharge were not investigated in the column studies. (Hübner et al. 2011)

Contact time is one of the most important factors and operating variables in biological drinking water treatment systems because it can significantly influence organic matter and contaminant removal. Depending on the objectives of biofiltration, the required contact time may be significantly different. Increasing temperature has been indicated to result in increased removal of biodegradable organic matter and some contaminants. This is expected because both mass transfer and biodegradation kinetics are favoured at higher water temperatures. On the other hand, mass transfer and microbial kinetics may be influenced also by the media type. Optimized filter media selection for biofilters has been concluded to depend on site-specific characteristics such as water quality and the plant's specific operational issues including for example contact time and operational pauses. In some bioreactors also backwashing is possible and necessary for sufficient operation. Results of several studies suggest that no linear relationship exists between attached biomass and removal of biodegradable organic matter. It has been concluded that the amount of biomass is not the limiting factor for organic carbon removal above some minimum level of biomass (Campos et al. 2002). (Urfer et al. 1997)

Grützmacher and Reuleaux (2011) introduced theoretical background on redox zoning in MAR systems and searched for applicable methods capable of controlling redox conditions. The main drivers for redox zonation can be controlled by most of the earlier discussed factors. Optimal redox zonation and residence times are necessary to gain maximum removal efficiency of redox-dependent substances. Many substances and substance groups show enhanced removal under oxic conditions or under anoxic to anaerobic conditions, but for many substances no redox dependency of removal can be observed. For example DOC, cyanotoxins, ammonium, some pesticides, many pharmaceuticals and x-ray contrast media, and some other trace organics show enhanced removal under oxic conditions. In addition, the mobilization of iron and manganese, as well as associated trace elements, like arsenic, is avoided under these conditions. Better removal efficiency under anoxic or anaerobic conditions is associated with nitrate, most disinfection by-products, some pesticides, some pharmaceuticals and x-ray-contrast media, and chlorinated hydrocarbons. Basically anoxic to anaerobic conditions should be avoided in MAR processes if substances showing enhanced removal under these conditions are not present in the source water. (Grützmacher and Reuleaux 2011)

Biological activity, residence time and the availability of oxidizing and reducing agents and nutrients are the main drivers for redox zonation, and they can be controlled by many natural, site-specific, design and operation-related variables including, for example, temperature, aquifer geochemistry, and distance between the infiltration pond and the production well. In MAR, variables and drivers affecting redox-zoning can be manipulated in two or three different zones, including the infiltration pond, the hyporheic zone and the subsurface passage. A summary of the main drivers for redox processes and the influencing variables is presented in Table 2.1. (Grützmacher and Reuleaux 2011)

Table 2.1 *Summary of drivers for redox processes and the influencing exogenous and decision variables in managed aquifer recharge (Grützmacher and Reuleaux 2011).*

topic	drivers	main redox-related process	influcencing exogenous and decision variables
hydro-geochemistry (infiltrating water, ambient groundwater and aquifer material)	availability of oxidizing agents (oxygen and nitrate)	respiration, denitrification	aquifer geochemistry (organic matter, mineral phases), temperature, natural gw recharge, presence & thickness of unsaturated zone, water table oscillations
	availability of reducing agents (organic matter and mineral phases)	reduction of oxygen and nitrate, pyrite oxidation	geochemistry of infiltration basin material, aquifer geochemistry, mixing with ambient groundwater
biological activity	bacteria in the subsurface	respiration	temperature, nutrients (C, N, P, K, DOC), pH
	algae in the infiltration pond	DOC consumption, O_2 production	temperature, sunlight, nutrients
hydraulic characteristics	residence time	reaction kinetics of redox reactions	well field geometry, distance pond to production well, pumping rate, hydraulic conductivity of aquifer and clogging layer, presence & thickness of unsaturated zone, temperature (water viscosity), retardation coefficient

The pretreatment and the infiltration pond can be used to control the composition of the source water. Controlled growth of algae in the infiltration pond may be used to remove DOC but the risks of the occurrence of cyanobacteria and possibly cyanotoxins should be taken into account. Bacterial activity could be increased with the addition of specific compounds, for example nutrients or organic content, and the water level in the pond could be used to control infiltration rates. Temperature of the water and sunlight intake can be controlled by constructing indoor-ponds. The strongest biochemical activity occurs commonly in the hyporheic zone, which could be manipulated by creating one or two meters of artificial sediments in the zone with desired characteristics. This way for example adsorption of target substrates could be enhanced. This leads to increased residence time and possibility for degradation. In addition to controlled hydraulic conditions, also DOC, chemical conditions, and temperature could be manipulated but the manipulation of these factors may constitute a very complex approach. Influencing the process during subsurface passage is possible via wells along the flow path. Wells can be used for substrate transferring into groundwater, monitoring, controlling the

hydraulic regime, and manipulating hydraulic conductivities through precipitation. (Grützmacher and Reuleaux 2011)

Several applications have already been developed to affect biological activity and hydro-geochemistry during aquifer recharge. They are based on the idea of changing chemical conditions within the subsurface, usually to intensify or trigger purification processes. Increased bacterial activity and suitable living conditions have been achieved by injection of gaseous oxygen, aerated water, anaerobic water or otherwise manipulated water into aquifer according to treatment objectives. (Grützmacher and Reuleaux 2011)

3. MATERIALS AND METHODS

3.1. Virttaankangas AGR site description

Virttaankangas AGR site has been planned to supply drinking water for the needs of the Turku region in southwest Finland, which has suffered from quality problems in raw water and insufficient water reserves. The Virttaankangas formation belongs to the Säkylänharju-Virttaankangas glaciofluvial complex, which is part of a large esker system in south-west Finland. The Virttaankangas complex covers about 80 km^2 and the total thickness of unconsolidated sediments generally varies from 20 to 50 m, but reaches 90 m locally in a bedrock fault zone. Three distinct aquifer units are recognized in the Virttaankangas formation, and the artificial groundwater production at the site takes place in an unconfined esker core aquifer, composed of glaciofluvial medium sand and gravel. (Artimo et al. 2007; Kortelainen and Karhu 2009)

Before the beginning of the actual drinking water production at the AGR site, an experimenting period was performed to simulate groundwater flow rates in production conditions. The experimenting period was performed between 15.9.2010 and 17.3.2011, and it comprised pretreated river water infiltration into a pristine aquifer at the eastern part of the Virttaankangas AGR site.

Figure 3.1 presents the eastern part of the AGR site, consisting of production well area VO50 and the infiltration areas IA401, IA500, IA501 and IA503. In the eastern operating area groundwater is flowing southeast parallel to the Säkylänharju-Virttaankangas complex. Natural groundwater flow from infiltration area IA500 to production well K51 has been discovered to be slow, taking approximately 8 months. (Artimo et al. 2007) The experimental period offered a rare opportunity for MAR research, and it was used to investigate and monitor the chemical and microbial changes during the start-up of a new AGR plant. The effects of river water infiltration were monitored along the aquifer flow path from the infiltration area IA401 to production well area VO50 between September 2010 and March 2011.

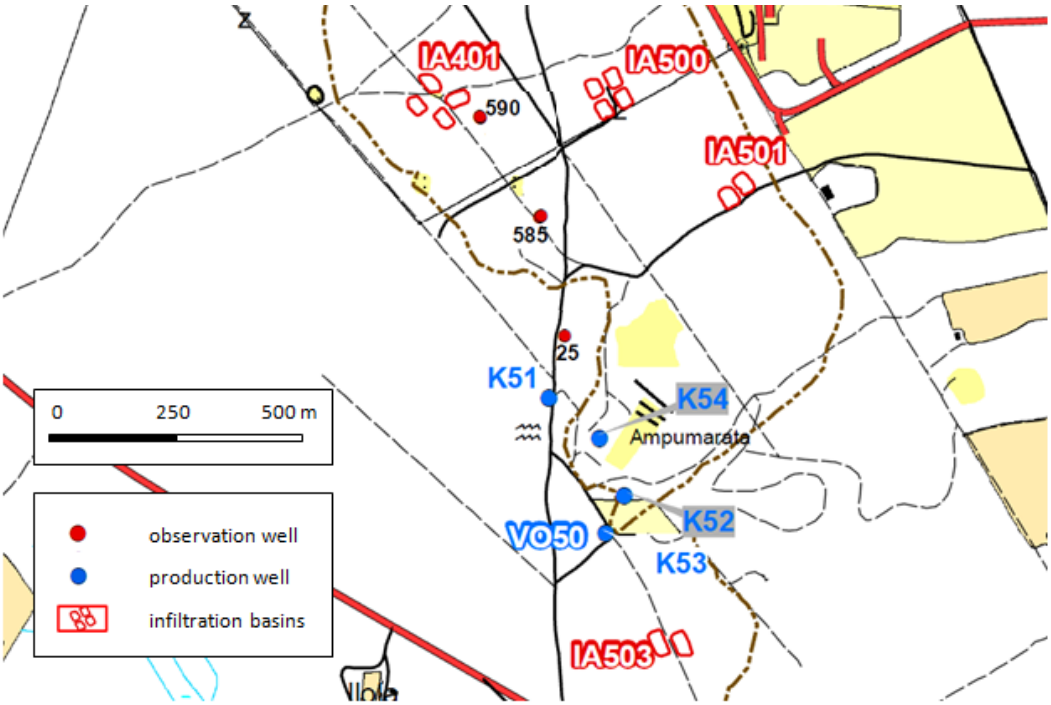

Figure 3.1. *Eastern part of Virttaankangas AGR site including production well area VO50, infiltration areas IA401, IA500, IA501 and IA503, and observation wells 590, 585 and 25. (Modified from Turun Seudun Vesi Oy 2011)*

Raw water for the artificial recharge is gained from the River Kokemäenjoki, nearby Huittinen in south-west Finland, approximately 30 km from the AGR site. The surface water is pretreated before it is pumped to a water reservoir at the AGR site. The pretreatment includes microstraining which removes large-sized solid matter, followed by chemical precipitation with polyaluminium chloride, and filtration through a sand-anthracite filter media. At Virttaankangas AGR plant, the pretreated water is conveyed gravitationally or by pumping from the water reservoir to the infiltration basins. Each infiltration area includes two or four excavated infiltration basins, each basin being approximately 500 m^2 in area. During the experimenting period at the eastern site, the average river water infiltration rate was 19 276 m^3/d, and the water pumped from the production wells was on average 18 952 m^3/d. (Turun Seudun Vesi Oy 2011; Turku Region Water Ltd 2012)

3.2. Sampling information and performed analyses

Samplings for the chemical and microbial monitoring were performed four times between 16.9.2010 and 2.3.2011 at the eastern Virttaankangas AGR site. Water samples were taken from the incoming water (INC), and observation wells 590, 585 and 25. The first sampling did not include sampling of the incoming water, and river water was additionally sampled at the last sampling. Attached phase samples were taken from the observation wells 590, 585 and 25. At the first sampling, high density polyethylene (HDPE) biofilm collector slides (Verbio inc.) were installed into each observation well to the sampling depth for the future attached phase sampling. Consequently, attached

phase sampling was performed for the first time at the second sampling in October 2010. The observation wells included in the sampling program are listed in Table 3.1 with the sampling depths. The locations of the observation wells at the eastern Virttaankangas AGR site are presented in Figure 3.1.

Table 3.1. *Observation wells included in the sampling program during river water infiltration. Groundwater surface depths and sampling depths are measured from the top of the observation well.*

Well	Total length of the observation well (m)	Groundwater surface depth 09/2010 (m)	Sampling depth (m)
590	32	18,26	25,0
585	78	17,14	35,0
25	31	15,08	25,0

The basic chemical monitoring of each water sample included pH, temperature, redox potential and dissolved oxygen (DO) measurements on-site, and TOC, DOC, ultraviolet absorbance (UVA) and NOM molecular size distribution analyses in the laboratory. The basic microbial monitoring of each water phase and attached phase samples included bacterial cell counts and microbial community analysis. In addition to the basic chemical and microbial monitoring, additional analyses were also performed. These included RNA assays and activity assays of both water phase and attached phase samples, and inorganic analyses of nitrate (NO_3^-), nitrite (NO_2^-), ammonium nitrogen (NH_4-N) and total phosphorous (P) in water samples. River water samples were not included in the activity assay and inorganic analyses. The sampling schedule, duration of river water infiltration from the beginning of the experimental period, and additional sampling information are listed in Table 3.2. Microbial communities in soil samples from observation well drillings were also analyzed to compare the results with attached phase microbial community of the biofilm collector slides.

Table 3.2. *Sampling dates, duration of river water infiltration, and additional samplings at Virttaankangas AGR site.*

Date	River water infiltration	Additional sampling information
16.09.2010	1 d	no INC and attached phase sampling
27.10.2010	42 d	-
21.12.2010	96 d	-
02.03.2011	167 d	river water sampling, RNA assay, activity assay, inorganic analyses of NO_3^-, NO_2^-, NH_4^- and total P

For each observation well, attached phase sampling for the microbial monitoring was performed before groundwater sampling. The biofilm collector slide holder was lifted from the observation well, and biofilm collector slides were removed from the holder with sterile tweezers and put into sterile 15 ml Falcon tubes. Water phase sampling and on-site measurements were performed after pumping stagnant groundwater from the observation wells for 20 minutes to gain representative samples. Incoming water sample was taken from an infiltration basin in October, but the next samplings were performed from the water reservoir due to difficult sampling conditions outdoors. River water sampling was performed at the raw water intake plant.

Water samples for microbial community analysis were taken into autoclaved 2 l glass bottles, and the water samples for bacterial cell counts were taken into sterile 50 ml Falcon tubes. Samples for the basic chemical monitoring were taken into acid washed 200 ml plastic bottles, which were filled to the brim. For the TOC samples, 40 µl of 50% H_2SO_4 was added into the bottles before sampling. Samples for the inorganic analyses were taken into a 1 l plastic bottle. The sampling method for the RNA assay is described in section 3.10, and for the activity assay in section 3.11. The samples were transported and stored in the dark and in cold temperature (+4°C) until further processing. The performed measurements and analyses are described in the following chapters.

3.3. On-site measurements

Water pH, temperature, redox potential, and dissolved oxygen were measured at the sampling site. Measurements were done with HQ30d portable meter (Hach) connected with a pH electrode pHC301, a redox electrode MTC301 or a luminescent dissolved oxygen probe LDO101 (Hach). Redox and pH electrodes had a built-in temperature sensor. Temperature, pH and redox potential were measured from a vessel filled with ground water sample. Dissolved oxygen was measured by leading ground water to flow directly from the pump to a small chamber connected with the DO probe. The validity of pH measurements from December 2010 and March 2011 is questionable because the electrode was functioning inaccurately probably due to the cold weather conditions and high temperature differences during the samplings.

3.4. Organic carbon analyses

All vessels used for sampling and analyses of organic carbon were acid washed with 1 M HCl overnight and rinsed with MilliQ. The samples were analyzed or frozen for later analysis within 24 hours after sampling. DOC samples were prepared by filtering groundwater samples through 0,45 µm Millex-LCR PTFE membrane filters (Millipore). Before filtering, the membrane filters were rinsed with 40 ml of 60°C MilliQ.

Concentrations of TOC and DOC were determined according to standard SFS-EN 1484 (1997) using TOC-V_{CPH} analyzer (Shimadzu) with TOC standard catalyst. TOC and DOC were measured with NPOC (non-purgeable organic carbon) analysis method using injection volume of 150 µl. All analyses were done for parallel samples, including two different parallel standard solutions. Filtered and unfiltered MilliQ samples were also included to every analysis to determine the carbon content of the filtrate.

3.5. Ultraviolet absorbance

Ultraviolet absorbance was measured from parallel TOC and DOC samples at 254 nm wavelength with Shimadzu UV-1601 UV-Visible spectrophotometer. The results were calculated as the average from the results of two parallel samples. To estimate the amount of aromatic carbon in the samples, the specific ultraviolet absorbance ($SUVA_{254}$) was calculated by dividing the UV absorbance of the sample (UVA_{254}) by the DOC concentration of the sample (Equation 3.1). The aromaticity percentage of a DOC sample was calculated with Equation 3.2.

$$SUVA_{254} = \frac{UVA_{254}}{DOC} \quad (3.1)$$

$$Aromaticity\ (\%) = 527 \cdot SUVA_{254} + 2{,}8 \quad (3.2)$$

3.6. Molecular size distribution of NOM

The molecular size distribution of dissolved organic matter was determined from the DOC samples using high pressure liquid chromatography (HPLC). Within 24 hours after sampling, parallel DOC samples of 1 ml were pipetted into HPLC vials and stored in -20°C until the analysis. The analysis was done using Hewlett Packard 1100 Series high pressure liquid chromatograph with a Diode Array UV-detector at 254 nm wavelength. Silica based TSKgel G3000SWXL SEC column (7,8 mm ID × 30,0 cm L, Tosoh Bioscience) was used at 30°C in the column temperature control oven. 0,01 M sodium acetate was used as carrier solvent at a flow rate of 1 ml/min. The system was calibrated with acetone (58 Da) and polystyrene sulphonate standards of the following molecular weights (MW): 210, 4300, 6800, 13000, 17 000 Da (Fluka) and 1920 and 3620 Da (PSS Polymer Standards Service GmbH). The calibration curve of logMW versus retention time (in minutes) was described by Equation 3.3 (R^2 = 0,95).

$$y = -0{,}3117x + 5{,}7095 \quad (3.3)$$

3.7. Phosphorus and nitrogen analyses

Ammonium, nitrite, nitrate and total phosphorus were measured from the water samples taken in March 2011, excluding the river water sample. Analyses were done in the laboratory of Kokemäenjoen vesistön vesiensuojeluyhdistys ry (KVVY ry, Water Protection Association of Kokemäenjoki River) using standards SFS-EN ISO 13395 (1997, modified) for nitrate and nitrite, SFS-EN ISO 11732 (modified) for ammonium and SFS-EN ISO 6878 (modified) for total phosphorus.

3.8. Total cell counts

Microbial abundance was determined from both water and attached phase samples. The sample preparations were performed within 2 days after sampling. Cells were resuspended from a biofilm collector by placing the collector into a 50 ml Falcon-tube containing approximately 10 ml of small sterile glass beads. The tube was filled with approximately 40 ml of sterile 0,9% sodium chloride and shaken for 10 minutes in a rotation shaker at room temperature. Before filtering, the suspension was vortexed for 20 seconds.

Water samples and resuspended attached phase samples were filtered with sterile equipment through 0,2 µm polycarbonate membrane filters (Millipore), 25 mm in diameter. Two filtrations with different volumes were performed for the samples from each sampling point. The filtered sample volumes depended on the estimated cell density of the sample. The filtrated volumes for different sample types are presented in Table 3.3. The cells on the filters were stained with 4',6-diamidino-2-phenylindole (DAPI) by adding 1 ml of 1 mg/l DAPI solution into the filtrating tower for five minutes. After staining, the filters were placed on a microscopic glass, and a cover slip was carefully placed on the filters, both with a drop of Citifluor (Citifluor Ltd). The samples were stored in the dark at +4°C.

Table 3.3. *Filtrated sample volumes for different sample types.*

Sample type	**Filtrated volume**
Natural groundwater	20 and 25 ml
River water	5 and 10 ml
Pre-treated river water	10 and 15 ml
Artificial groundwater	15 and 20 ml
Resuspended attached phase sample	0,5 and 1 ml

The number of bacterial cells was counted from twenty randomly chosen spots within a know area using the Zeiss Axioscop 2 epifluorescence microscope (Zeiss). For the water samples the number of cells per volume was determined with Equation 3.4. The

number of cells per area was determined with Equation 3.5 for the resuspended attached phase samples. The final abundances for the samples were calculated as an average value from the two filtrations.

$$cells/ml = \frac{K_A \cdot C}{V_S} \quad (3.4)$$

$$cells/cm^2 = \frac{K_A \cdot C}{V_S} \cdot \frac{V_T}{A} \quad (3.5)$$

In Equations 3.4 and 3.5, K_A is the average amount of cells from twenty counts, C is a conversion factor, V_S is the filtrated sample volume in millilitres, V_T is the total volume of the resuspension solution (40 ml) and A is the area of the biofilm collector slide (8,4 cm^2). The value of the conversion factor C (Table 3.4) depends on the area used for the cell counts.

Table 3.4. *Conversion factors for the different areas used for the cell counts.*

Area	Conversion factor C
1x1	1460500
2x2	365113
3x3	162272
5x5	58418
10x10	14605

3.9. Bacterial community analyses

The bacterial community analysis included DNA extraction from the water samples, biofilm collectors and soil samples. Extracted DNA was amplified and the bacterial communities were profiled with length heterogeneity - polymerase chain reaction (LH-PCR). In the following sections the extraction procedures are described for the three different types of samples, followed by the description of PCR amplification and LH-PCR analysis. Extracted DNA and PCR products were stored at -20°C.

Water samples

The water samples were filtered with sterile equipment within a day after sampling through 0,2 µm polycarbonate filters (Whatman), 47 mm in diameter. Filtrated volumes varied from approximately 250 ml to 2000 ml, depending on the turbidity of the sample. The filters were placed on sterile Petri-dishes and stored at -20°C until DNA extraction. DNA extraction was done using PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc.), according to the given protocol.

Attached phase samples

DNA extraction for the attached phase samples was performed within a day after sampling. The cells from the biofilm collector slides were resuspended into sterile 0,1% sodium dodecyl sulphate (SDS) solution in 50 ml Falcon tubes. The collector slides were placed into Falcon tubes, which were then filled with the SDS solution until it covered the collector slide. The tubes were sonicated for three minutes at room temperature, and subsequently vortexed for 20 s. The collector slides were removed from the tubes with sterile tweezers, and the tubes were centrifuged for 40 minutes at 5000 × rcf at room temperature. The supernatant was removed carefully immediately after centrifugation by pipetting. Beads and the PowerBead solution from the PowerBead Tubes of PowerSoil DNA Isolation Kit were added into the Falcon tubes. The contents of the tubes were vortexed briefly and returned to the PowerBead Tubes. The extraction was continued according to PowerSoil DNA Isolation Kit protocol, but as exception to the protocol, the time for the horizontal vortexing was halved to five minutes.

Soil samples

The soil samples for the DNA extraction were taken from the observation well drillings from different depths. The soil samples from the drillings were divided into approximately 10 g fractions with sterile equipment. The fractions were put into sterile 15 ml Falcon-tubes and stored at -20°C until DNA extraction. DNA extraction was done according to given protocol, using the UltraClean Mega Soil DNA Isolation Kit (Mo Bio Laboratories, Inc.).

LH-PCR analysis

Fragments of the 16S ribosomal RNA gene were amplified from the DNA extracts using bacterial reverse primer 518R (5'-ATTACCGCGGCTGCTGG-3') and bacterial forward primer 27F mixture. The primer 27F mixture consisted 75% of unlabelled primer (5'-AGAGTTTGATCMTGGCTCAG-3') and 25% of IRD-700 labeled primer (5'-IRD-700-AGAGTTTGATCMTGGCTCAG-3'). The amplification resulted in PCR products approximately 500 base pairs (bp) in length. All the PCR amplifications were performed with a T3000 Thermocycler (Biometra). The template volumes were defined based on the strength of the bands on agarose gel in amplification testing with different template volumes. 4 µl of extracted DNA was used as a template for water samples and attached phase samples, and 2 to 10 µl of extracted DNA for the soil samples. A 25 µl PCR mixture contained 1×GoTaq reaction buffer (Promega), 0,4 mg/ml BSA (Fermentas), 0,2 mM of dNTPs (Fermentas), 0,3 µM of each primer (Oligomer Oy), 0,65 U GoTag polymerase (Promega), and PCR grade water (MP Biomedicals) to the final mixture volume. The PCR procedure included an initial denaturation step at 95°C for 5 minutes, followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 3 minutes. The final extension step was increased with 1 minute at 72°C.

Triple separate PCR amplifications were performed for each DNA extract, and the PCR products were verified by agarose gel electrophoresis. Agarose gel was made with 1% agarose (Sigma-Aldrich GmbH) dissolved with 1×TAE buffer (40 mM TRIS acetate, 1 mM EDTA, Sigma-Aldrich GmbH) and stained with 1×SYBR safe DNA gel stain (Invitrogen). 5 µl of PCR product mixed with 1 µl of 6×DNA Loading Dye (Fermentas) was loaded on the gel, and GeneRuler DNA Ladder Mix (Fermentas) was used to verify the length of the PCR products.

The triplicate PCR amplifications of each sample were mixed together to form the final product for the LH-PCR analysis. 0,05-4 µl of the pooled PCR product was mixed with 20 µl of loading mix (33% loading buffer, 10% 10×PCR buffer, 57% PCR grade water). The volume of the pooled PCR product depended on the intensity of the band on 1% agarose gel. Gel electrophoresis for the LH-PCR analysis was performed with a LI-COR 4300 DNA Analyzer (LI-COR Biosciences) using 6% Long Ranger polyacrylamide gel mix (Lonza Group Ltd). Size standards of 469, 528 and 545 bp, and duplicates of each sample were included in the LH-PCR analysis.

LH-PCR gel analysis was performed using the program GelCompar II version 4.6 (Applied Maths NV). The fluorescently labeled primer allows determination of the relative amounts of amplified sequences originating from different microorganisms, and the analysis results contained the lengths of detected bands (bp) and their relative quantities (%) in each sample. The band lengths were rounded up to enable the comparison of different samples causing a possible ±1 bp error in the results. The results of parallel samples were compared, and the more representative sample according to intensities and clarities of the bands was included in the results.

3.10. RNA assay

The sampling for the RNA assay was conducted on 2.3.2011. The aim of the assay was to compare the RNA bacterial community profiles of both water and attached phase samples against the corresponding DNA profiles. This way it is possible to determine the active members of the microbial communities from the members that are only present in the samples.

Duplicate water samples from the river water, incoming water, and observation wells 590, 585 and 25 were taken for the RNA assay. A single water sample for the RNA analysis requires 20 ml of sample water, and, thus, the following sampling procedure was performed four times at each sampling point to gain duplicate samples: 10 ml of sample water was added into sterile 50 ml Falcon tube which contained 20 ml of stabilizing reagent RNAprotect Bacteria Reagent (QIAGEN). The tubes used for measuring the 10 ml of sample water were made RNase-free by rinsing them with RNAprotect Bacteria Reagent. Likewise, duplicate attached phase samples from

observation wells 590, 585 and 25 were included in the assay. Two biofilm collector slides were needed for a single attached phase sample. The slides were put into a sterile 15 ml Falcon tube, which was filled with RNAprotect Bacteria Reagent and contained sterile glass beads in the bottom.

The RNA assay included RNA extraction from the water samples and biofilm collectors, followed by DNase treatment and reverse transcription (RT) of the extracted RNA. The RT-PCR product was further amplified for the LH-PCR analysis.

In order to capture the RNA for extraction, the stabilized samples were filtrated within the sampling day. Before filtration, the attached phase samples were shaken for 10 minutes in a rotation shaker at room temperature for detaching the biofilms from the collector slides. Prior to filtration of the samples, 0,2 µm polycarbonate membrane filters, 25 mm in diameter (Millipore), were rinsed once with RNAprotect Bacteria Reagent and twice with diethylpyrocarbonate (DEPC) -treated water. DEPC-treated water was prepared by incubating MilliQ with 0,1% (v/v) DEPC at 37°C for 12 hours followed by autoclaving to remove the DEPC. The samples were filtrated using sterile, RNase-free filter equipment and stored in Petri dishes at -80°C until RNA extraction. RNA extraction was done using the UltraClean Microbial RNA Isolation Kit (Mo Bio Laboratories, Inc.), according to the given protocol.

DNase treatment was performed in a 20 µl reaction containing 2 U DNase I enzyme (Fermentas), 1×reaction buffer with $MgCl_2$ (Fermentas) and 16 µl of extracted RNA by incubating reaction mixture at 37°C for 30 minutes, followed by 2 µl addition of 50 mM EDTA (Fermentas) and incubation at 65°C for 10 minutes.

Reverse transcription of DNase treated RNA into cDNA was performed in two steps, working on ice. 12,5 µl of DNase treated RNA was used as template in a 20 µl RT-PCR reaction mixture containing 5 µM of random hexamer primer (Fermentas), 0,5 mM of dNTPs (Fermentas), 1×RT buffer, 20 U of RiboLock RNase inhibitor (Fermentas) and 200 U of RevertAid Premium Reverse Transcriptase (Fermentas). First, the template RNA, primer and dNTPs were carefully mixed and incubated at 65°C for 5 minutes. Secondly, the RT buffer, RNase Inhibitor and RevertAid reverse transcriptase were added, and the mixture was incubated at 25°C for 10 minutes followed by 30 minutes at 50°C. Reaction was terminated by heating at 85°C for 5 minutes.

Amplification, sample preparation for LH-PCR and LH-PCR analysis were performed as described in section 3.9 (LH-PCR analysis). Triple separate PCR amplifications were performed from every sample using 6 µl of cDNA as a template.

3.11. Activity assay

Biofilm collectors from observation wells 590, 585 and 25, and water samples from the incoming water and observation wells 590, 585 and 25 were included in the leucine incorporation activity assay on 2.3.2011. As a small amino acid, leucine is easily assimilated by bacteria. Because approximately 50% of bacterial dry mass consists of proteins, leucine incorporation into bacterial proteins is a straight forward method for measuring bacterial biomass production. The leucine incorporation method can be used in a wide range of aquatic habitats, and an estimate of microbial production can be used as a general index of microbial activity. (Tuominen 1995; Fischer and Pusch 1999; Kirchman 2001)

Triplicate samples were taken from each sampling location: 10 ml of water and 1 biofilm collector were placed in sterile 15 ml Falcon tubes with 10 nM of C^{14}-leucine (Perkin Elmer, specific activity 318 µCi/µmol). For the determination of background radiation, control samples were prepared by immediately adding another triplicate of samples with 5% (v/v) TCA to kill the cells and thus prevent any leucine uptake.

The samples were incubated in an electric cooler below +5°C for 3,5 hours. The temperature was set near the water temperature range measured from the observation wells (1,5-5,7°C). Following incubation, leucine incorporation was stopped by the addition of 5% (v/v) TCA to kill the cells. The killed samples were stored at +4°C for 75 days until radio assay.

For the radio assay, the water samples were filtered through 25 mm Ø, 0,2 µm white polycarbonate filters (Millipore) and rinsed three times with ice-cold 5% TCA and once with ice-cold 100% ethanol. The filters were placed into scintillation vials and dissolved by incubating the vials for one hour at +60°C, in 1 ml of an aqueous based solubilizer SOLVABLE (PerkinElmer). The biofilm collectors were rinsed with ice-cold 5% TCA to remove excess leucine, placed into fresh 15 ml Falcon tubes and incubated by shaking the tubes horizontally (150 rpm) at +60°C for one hour with 2 ml of SOLVABLE. An aliquot (1 ml) of the dissolved biofilm sample was dispensed into a scintillation vial for radio assay.

10 ml of OptiPhase Hisafe scintillation cocktail (Fisons Chemicals) was added into the vials and the samples were radio assayed with a 1217 Rackbeta Liquid Scintillation Counter (Wallac Scintillation Products). The activities were measured in counts per minute (cpm). For the final results, the mean of the background counts was subtracted from the means of the sample counts.

In order to enable comparison of activities between the attached and water phase as well as between different sampling locations, total cell numbers in the 10 ml water samples and on the area of 8,4 cm^2 of the biofilm collectors needed to be determined. Cell counts could not be conducted for the radiolabeled samples directly and, thus, cell numbers were obtained using corresponding routine monitoring DAPI-counts (see section 3.8).

The average counts obtained from the radio assay were converted to leucine uptake rates per hour per cell using Equation 3.6.

$$mmolLeu/cell/h = \frac{(avcpm_{sample} - avcpm_{control}) \cdot 4{,}5 \cdot 10^{-13}}{SA \cdot t \cdot totalcells} \qquad (3.6)$$

where SA = specific activity of leucine (Ci/mmol)

t = incubation time (h)

$4{,}5 \cdot 10^{-13}$ = number of curies per cpm

(1 Ci = $2{,}22 \cdot 10^{12}$ cpm)

(Adapted from Bell 1993)

Standard deviations were calculated for the leucine uptake rates in order to determine the significance of any difference between the samples and the validity of the measurements.

4. RESULTS AND DISCUSSION

4.1. On-site results

The results of measured pH-values during river water infiltration are presented in Figure 4.1. The results show that the pH values were stable in Virttaankangas aquifer ranging from 8,1 to 9 during the whole sampling period. Based on concurrent pH measurements by Turku Region Water Ltd, the pH of the infiltrated water (INC) can be assumed to be lower than presented in Figure 4.1. The high pH-readings measured from the incoming water are probably due to inaccurate functioning of the pH electrode followed from cold weather conditions and high temperature differences between the samples. Assuming that the pH of infiltrated water was in average approximately 7, the infiltrated water did not seem to affect the pH in the aquifer during the river water infiltration. Instead, the pH value of the infiltrated water immediately rose to over 8 in the aquifer.

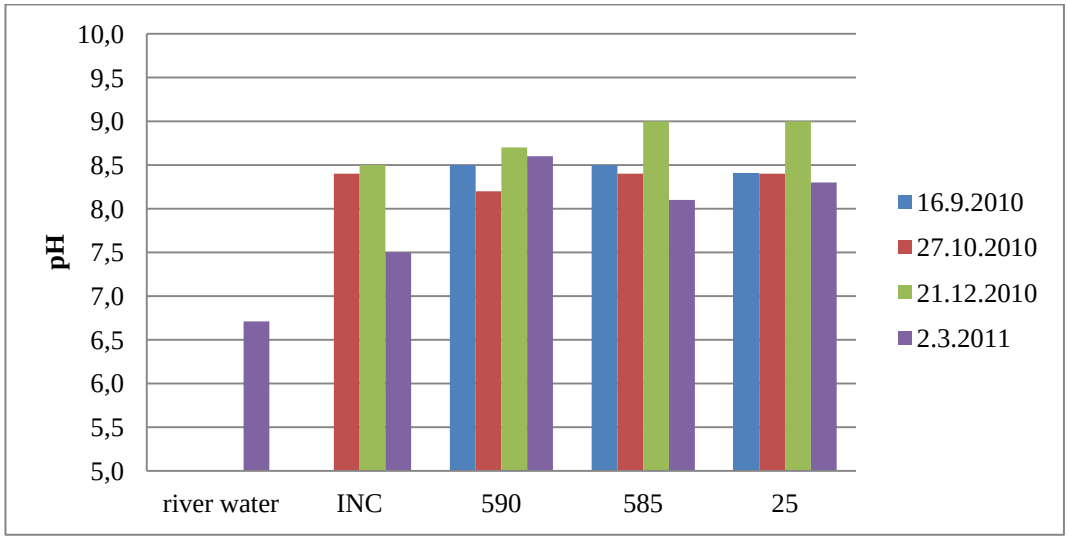

Figure 4.1. *PH-values in Virttaankangas aquifer during river water infiltration. The pH for infiltrated water is assumed to be lower due to cold weather conditions and high temperature differences between the samples that caused inaccurate functioning of the pH electrode.*

The pH values measured from the observation wells were in normal range. At the eastern part of the AGR site, the pH values have been reported to vary between 8 and 10, generally between 8,5 and 9 (Artimo et al. 2007). The high pH values exceeding 9 are unusual in unconsolidated glacigenic aquifers in Finland, where the pH values are

normally averaging 6,4 in silicate-rich shallow groundwater formations in crystalline bedrock areas. The high pH values in Virttaankangas aquifer indicated carbonate-bearing aquifer material, and the sediments have been discovered to contain small amounts of fine grained, dispersed calcite. Reactions between dissolved CO_2 and calcite have a high tendency to increase the pH of local groundwaters, and the high pH values of groundwater are related to the dissolution of calcite into groundwater. (Kortelainen et al. 2007; Kortelainen and Karhu 2009) The dissolution of calcite explains also the raise of the infiltrated water pH.

Water temperatures in Virttaankangas aquifer during river water infiltration are presented in Figure 4.2. The measured temperatures ranged from 0,3°C to 8,3°C. The warmest temperatures in the aquifer were measured in September 2010 when the water temperature was approximately 8°C in all observation wells.

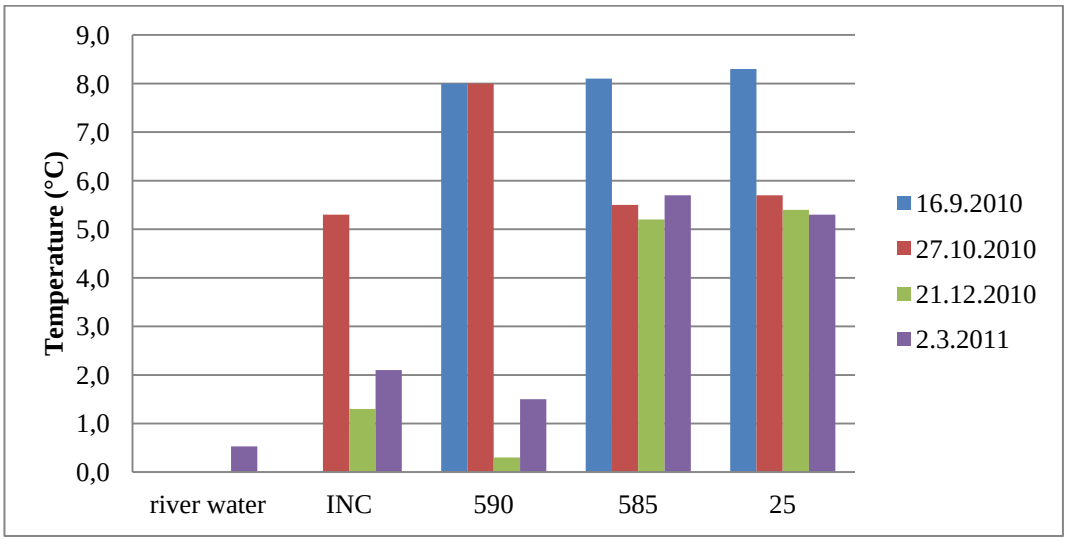

Figure 4.2. *Water temperatures in Virttaankangas aquifer during the sampling period.*

In October 2010, the water temperature in observation well 590 stayed at the same level as in September 2010 probably due to the warm temperature of the infiltrated water. In December 2010 and March 2011, the temperature of the infiltrated water significantly affected the water temperature in observation well 590. In December 2010 the groundwater temperature had decreased drastically to 0,3°C and was still under 2°C in March 2011. In December 2010 and March 2011, the temperature of infiltrated water was measured from the water reservoir, and this resulted in higher temperatures of infiltrated water compared to the temperatures in observation well 590. In winter, the water temperature decreases close to 0°C in the shallow infiltration ponds, which explains the decreased water temperature in observation well 590.

In comparison to observation well 590, the water temperature in both observation wells 585 and 25 had decreased from 8°C to approximately 5,5°C already in October 2010 and stayed stable until March 2011. The river water flow did not seem to affect the groundwater temperatures in these two observation wells. The measured temperatures were in the same range as measured earlier in the Virttaankangas aquifer, ranging from under 4°C to over 8°C (Artimo et al. 2007; Kortelainen and Karhu 2009).

Dissolved oxygen concentrations in river water, infiltrated water and in the aquifer are presented in Figure 4.3. In the aquifer, the DO concentrations were high and varied between 9,8 and 13,5 mg/l. The DO concentrations of the infiltrated water and river water were at similar level as in the aquifer; 11,2-12,6 mg/l.

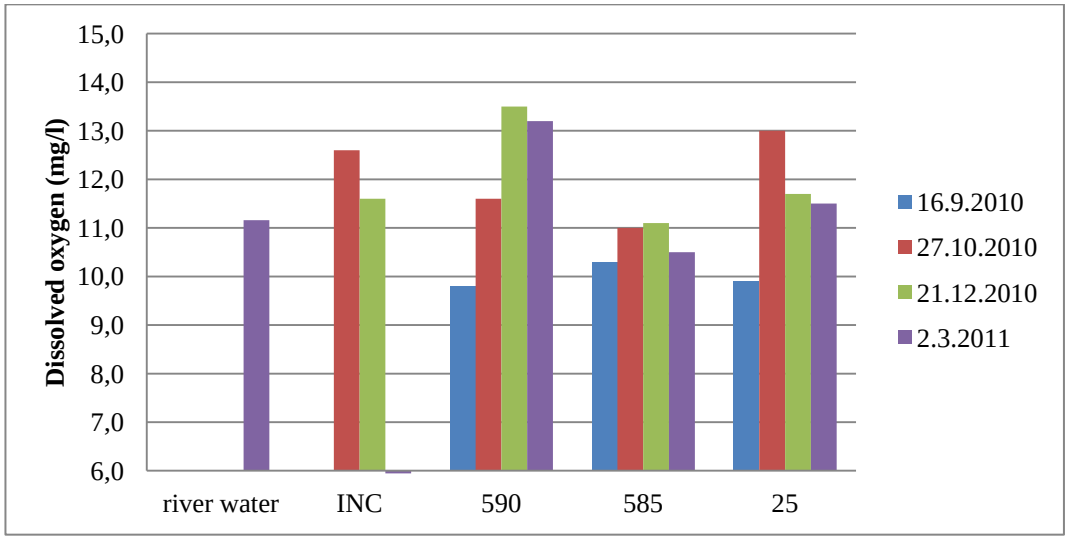

Figure 4.3. *Dissolved oxygen concentrations in Virttaankangas aquifer during the sampling period.*

During the sampling period, lowest DO concentrations in each observation well were measured in September 2010, when the groundwater temperature was highest. At the last three samplings, higher DO concentration appeared with the lower groundwater temperatures. The results are in agreement with the fact that the solubility of oxygen in water increases in decreasing temperatures (Karttunen and Tuhkanen 2003). Largest variations in DO (9,8-13,5 mg/l) were observed in observation well 590, where the temperature variations were also high. Between October 2010 and March 2011, the DO concentrations in observation well 590 are influenced by the relatively high DO concentrations and low temperatures of the infiltrated water. The DO concentrations in observation well 585 stayed relatively stable (10,3-11,1 mg/l) during the sampling period. The increase in DO concentration with the temperature drop between September and October 2010 was more apparent in observation well 25. The DO concentrations in the aquifer were in the same range as reported earlier from the eastern part of the AGR site (7,8-14,3 mg/l) (Artimo et al. 2007; Kortelainen and Karhu 2009).

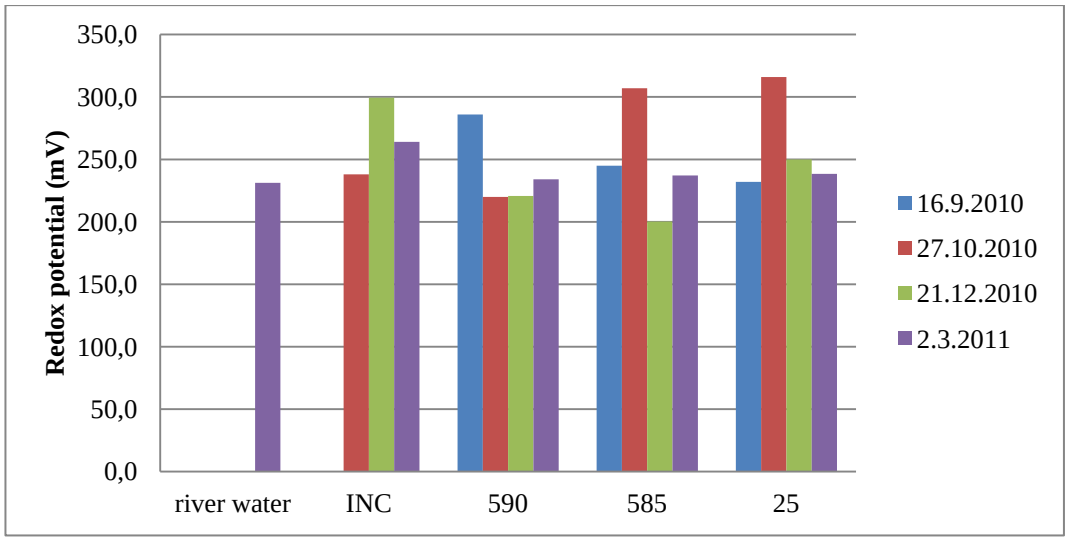

Figure 4.4. *Redox potentials in Virttaankangas aquifer during the sampling period.*

Redox potentials (Figure 4.4) in the aquifer varied between 200 mV and 316 mV. The redox potentials in the infiltrated water and the river water were in the same range with the values in the aquifer, between 231 mV and 299 mV, indicating that the infiltrated water did not affect the redox potential in the aquifer. The values above 200 mV indicate that the aquifer is aerobic and that oxygen is used as an electron acceptor during respiration (Maeng et al. 2010). This is also in agreement with the high DO concentrations in the aquifer and infiltrated water.

4.2. Organic carbon analyses

The measured TOC and DOC concentrations of water samples are presented in Table 4.1. According to the results, the DOC concentrations represented 97-100% of TOC concentrations in the aquifer and 94-100% in the river water and infiltrated water.

Table 4.1. *TOC and DOC results of groundwater samples during the river water infiltration.*

Sampling location	Sample	16.9.2010	27.10.2010	21.12.2010	2.3.2011
river water	TOC mg/l				9,0
	DOC mg/l				9,1
INC	TOC mg/l		5,4	5,1	4,6
	DOC mg/l		5,1	5,1	4,5
590	TOC mg/l	0,5	4,6	4,7	4,3
	DOC mg/l	0,5	4,7	4,7	4,4
585	TOC mg/l	0,4	0,5	0,6	0,9
	DOC mg/l	0,4	0,4	0,6	1,2
25	TOC mg/l	0,4	0,5	0,8	3,5
	DOC mg/l	0,7	0,6	0,8	3,4

Few DOC measurements resulted in higher concentrations when compared with the corresponding TOC concentrations. This is likely due to carbon detachment from filters or vials despite of acid washing of the equipment and rinsing of the filters. The DOC concentrations in water samples during the river water infiltration are presented in Figure 4.5.

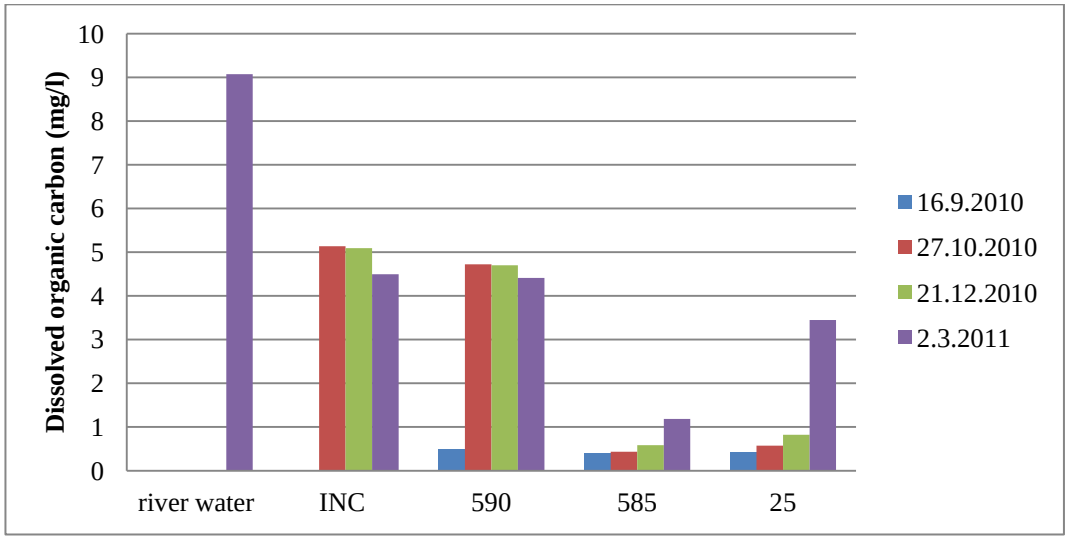

Figure 4.5. *DOC results at Virttaankangas during river water infiltration.*

According to the DOC results in March 2011, the pretreatment process halved the DOC concentration in river water. During the river water infiltration, the DOC concentration of infiltrated water stayed stabile, varying from 4,5 mg/l to 5,1 mg/l. The intrusion of infiltrated water into the aquifer is evident from the DOC results. In October 2010 the DOC concentration in observation well 590 had increased from 0,5 mg/l to 4,7 mg/l and stayed fairly stable at this level until the end of the sampling period.

The DOC concentration of the natural groundwater (September 2010) in Virttaankangas aquifer was approximately 0,5 mg/l. In December 2010, a slight DOC concentration increase from the natural level to 0,6-0,8 mg/l could be observed in both observation wells 585 and 25. In March 2011 the intrusion of the infiltrated water was more evident, and the DOC concentrations increased to 1,2 mg/l and 3,4 mg/l in observation wells 585 and 25, respectively. The results indicate that the infiltrated water reached both observation wells 585 and 25 approximately at the same time after 3 months of retention time. However, based on these results it seems that more infiltrated water had reached observation well 25.

In March 2011, the DOC result from observation well 25 is inconsistent with other data obtained from the site. Oxygen isotope data for March 2011 (provided by Turku Region Water Ltd) suggested that the share of infiltrated water was 44% and 34% in observation wells 585 and 25, respectively. Also, concurrent measurements (by Turku

Region Water Ltd, using S::can fluorometer) from an observation well adjacent to 25 returned much lower DOC concentrations (~1,4 mg/l on average). These data suggest that the increased DOC concentration in observation well 25 could be an anomaly caused by water channeling or another localized effect.

The Virttaankangas complex consists of five hydrogeological units: till, glaciofluvial coarse unit, glaciofluvial fine unit, silt and clay, and littoral sand. The coarse unit consists mainly of coarse sand and gravel, and has a good hydraulic conductivity. Therefore the major AGR operations are placed in this unit or in the immediate surroundings. The glaciofluvial fine unit surrounds the coarse unit, and some of the infiltrated water flows also in this unit. Significant differences in groundwater flow rates and volumes have been observed between the five hydrogeological units. The flow rate in the fine unit is slow compared with the coarse unit which forms a fast groundwater flow path. (Artimo et al. 2007)

The groundwater flow through different hydrogeological units could explain the difference in DOC results in observation wells 585 and 25. This would also indicate that the flow paths from the infiltration area IA401 to the two observation wells are different. The results of tracer test performed at the eastern AGR site between October 2002 and August 2003 indicated that different flow paths exist in the Virttaankangas esker (Artimo et al. 2007).

The results suggest that the system was still in the process of getting filled with the infiltrated water between December 2010 and March 2011. If the trend since December would have continued, the DOC concentrations would have probably increased further in observation well 585 and possibly in observation well 25 at the same time as the system got filled with infiltrated water. Within the framework of this study, it is impossible to say if the AGR system was stabilized in March 2011, to what extent the water in the two wells consisted of the infiltrated water, and if the situation in March would also describe the condition in drinking water production situation. Ignoring these drawbacks, the DOC reduction in March 2011 was 73% and 24% between the infiltration area IA401 and observation wells 585 and 25, respectively. The calculated DOC reductions between the infiltration area IA401 and observation well 590 were low, being 8%, 7,7%, and 1,8% in October 2010, December 2010, and March 2011, respectively.

Sorption, biodegradation and dilution with natural ground water are assumed to be the main NOM removal processes in AGR. Water in observation well 590 most likely consisted mainly of the infiltrated water, and, thus, some DOC removal by biodegradation and adsorption has can be assumed to have occurred between the infiltration basins and observation well 590. In observation wells 585 and 25, the role of dilution with native ground water in DOC reduction clearly becomes more important.

To determine to what extent the reduction is attributed to biodegradation, adsorption, and dilution, more detailed studies would be needed.

Although it is commonly reported that TOC and DOC removal dominantly occurs within the first few meters or in the beginning of infiltration (Maeng et al. 2011b), Helmisaari et al. (2006) reported that most of TOC reduction took place in the groundwater zone at five Finnish AGR sites. The low DOC removal rates in observation well 590 would be in accordance with the results of Helmisaari et al. (2006). In the study, raw water TOC concentrations varied between 5,7 mg/l and 10,8 mg/l, and the concentrations in extracted groundwater were between 1,1 mg/l and 2,1 mg/l. The retention times varied between 16-103 days and the flow path lengths were 160-1300 m. Including the effect of dilution with natural groundwater, the results showed 66-84% TOC removal. (Helmisaari et al. 2006) Compared to the other studied Finnish AGR sites, the DOC reduction in March 2011 was still low in observation well 25. In observation well 585, the DOC reduction was at similar level with other Finnish AGR sites, but the uncertainty of the infiltration process in March has to be taken into account. The low DOC concentration is probably due to low volume of infiltrated water in observation well 585.

It is difficult to hypothesize how fast enrichment of microorganisms and initiation of efficient biodegradation processes take place in a new AGR plant. In a pilot-scale AGR simulation, a good TOC reduction by approximately 85% was observed within 90 days after the start of infiltration in a biologically unadapted sand column (Kolehmainen et al. 2009b). On the other hand, low DOC reductions of approximately 34% have also been reported still after decades of infiltration at an AGR site (Grünheid et al. 2005). The large differences in NOM attenuation reported in different MAR sites and studies point out the site specificity of MAR processes.

To determine the sustainability of NOM attenuation in Virttaankangas aquifer, the NOM removal methods would have to be determined, and, if possible, quantified. One possibility for this are isotope methods, which can provide more information about NOM removal in AGR applications. The isotope ratios of oxygen and hydrogen can be used to calculate the mixing ratios between local groundwater and infiltrated water, and this way the influence of dilution to NOM removal can be estimated. (Kortelainen 2001) The method was used at Tuusula Waterworks AGR site in southern Finland, and the results showed that 14% of DOC was reduced by dilution during infiltration and that 52% of the extracted groundwater composed of natural groundwater. At the same AGR site, stable inorganic carbon isotope (δ13C) method was used to quantify DOC biodegradation (44%) and adsorption (23%). (Kortelainen and Karhu 2006) The δ13C method was also used to quantify DOC mineralization (32-52% of DOC removal) in pilot-scale AGR simulation by Kolehmainen et al. (2009b).

4.3. Ultraviolet absorbance

The ultraviolet absorbance of filtrated water samples are presented in Figure 4.6. In the pristine aquifer, the UV absorbance at 254 nm was approximately 0,006 in observation well 590 and 0,003 in observation wells 585 and 25. Absorbance of infiltrated water stayed fairly stable during the river water infiltration period varying between 0,086 and 0,095. In October 2010 the UV absorbance in observation well 590 had increased significantly and stayed stable at approximately 0,085 until March 2011. In observation wells 585 and 25, absorbance values stayed at the pristine aquifer level until December 2010. In March 2011 the values were affected by the infiltrated water and had increased to 0,019 and 0,060 respectively. In March 2011 the UV-absorbance of river water was 0,282 being over three times higher compared to the absorbance of infiltrated water produced after pretreatment.

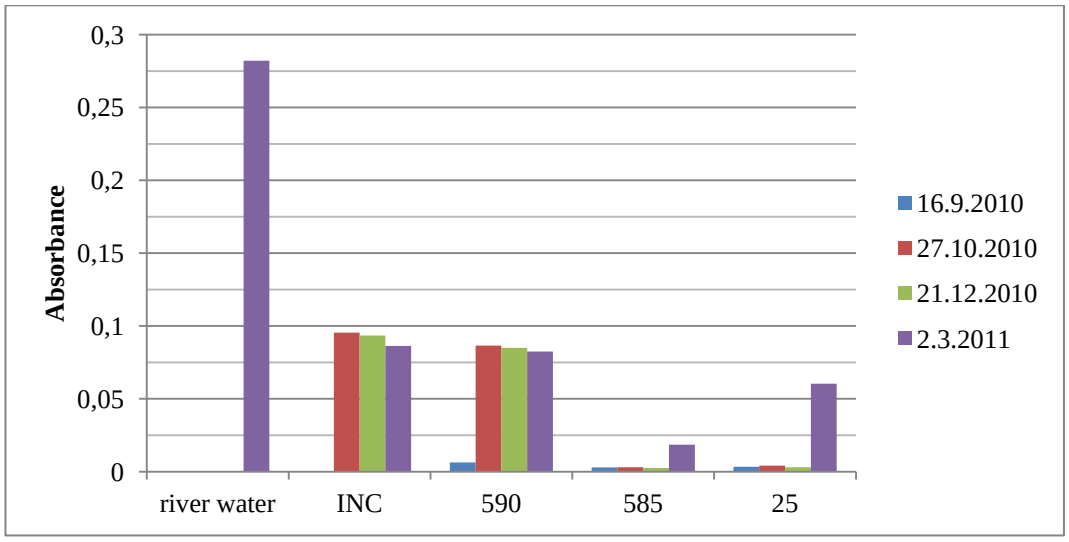

Figure 4.6. *Ultraviolet absorbance of DOC samples at 254 nm wavelength during river water infiltration.*

The aromaticity percentage of filtrated water samples were calculated with Equation 3.2, and the results are shown in Figure 4.7. Aromaticity in river water was 19% in March 2011. Pretreatment of river water produced water having stable aromaticity of 12,7% in average during the whole sampling period. In the pristine aquifer, water from observation well 590 had 9,5% aromaticity which was slightly higher compared to the approximately 6% aromaticity in observation wells 585 and 25. In October 2010 the aromaticity in observation well 590 had risen permanently to the same level with the infiltrated water (12,5%), correlating with the UV absorbance and DOC results. The aromaticity percentage dropped slightly in observation wells 585 and 25 in December 2010 despite the increased DOC concentrations. In March 2011 the aromaticity percentages had increased to 11% and 12%, respectively.

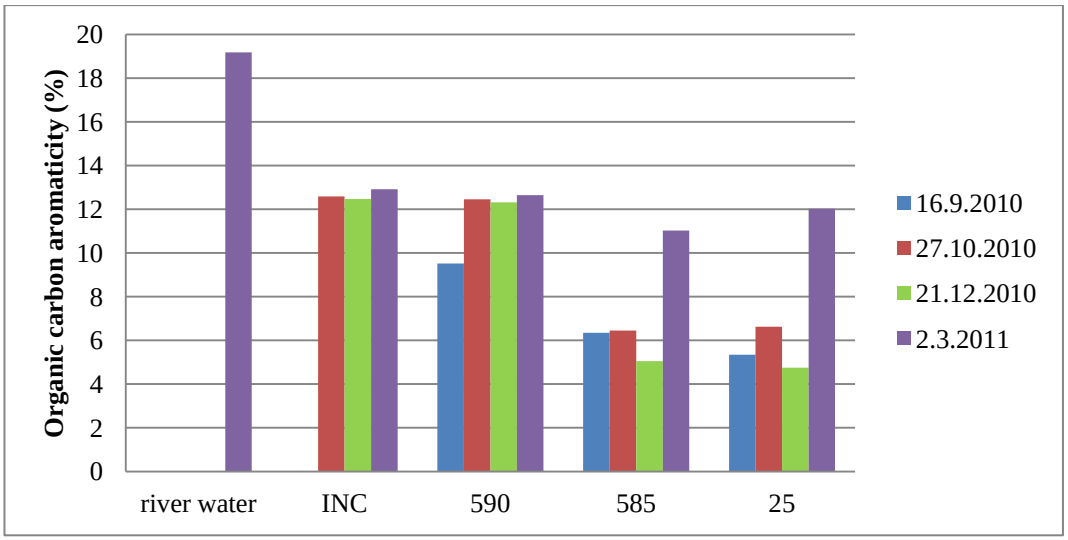

Figure 4.7. *NOM aromaticity in filtrated water samples during the sampling period.*

The slightly higher aromaticity of natural ground water in observation well 590 indicates local differences in the water quality within the aquifer. Preferential sorption and decreased mobility of aromatic organic matter has been demonstrated by several studies (Tufenkji et al. 2002). On the other hand, soil properties have a major influence on groundwater quality (Karttunen and Tuhkanen 2003), and therefore the local differences in water aromaticity may result from differences in soil properties, sorption within the flow path, and dilution in aquifer and with infiltrating rain water. The same level of aromaticity in infiltrated water and observation well 590 from December 2010 until March 2011 indicates that no aromatic organic matter was removed either by biodegradation or adsorption between the infiltration basins and the observation well.

In December 2010, the minor drop in aromaticity despite of the slight increase of DOC concentration in observation wells 585 and 25 can probably be explained by natural fluctuation in groundwater or by errors in measurement of low absorbance values. Despite of decreased mobility of aromatic, high molecular weight compounds, it would be fairly improbable that the intrusion of the infiltrated water would decrease the aromaticity of natural groundwater. In March 2011 the aromaticity in the two wells had increased nearly to the same level with the infiltrating water. The increase in aromaticity was higher in relation to the increase of DOC concentrations: in observation wells 585 and 25 the aromaticity was 85% and 93% of that in infiltrated water, respectively, whereas DOC was approximately 20% and 75% of the DOC in infiltrated water, respectively. In MAR processes, an increase in relative aromaticity is commonly attributed to preferential removal non-aromatic organic matter (Maeng et al. 2011b). On the other hand, disequilibrium between infiltrating water and native groundwater causes sorption and ion exchange (Farnsworth and Hering 2011), and since the infiltrated water was still filling the system, desorption of organic matter may be the reason for the relatively high aromaticity detected in observation wells 585 and 25.

4.4. Molecular size distribution of NOM

Figure 4.8 presents the molecular size distribution of NOM in infiltrated water and groundwater samples from October 2010 to March 2011. High pressure size exclusion chromatography (HPSEC) resulted in 6 size fractions in infiltrated water and groundwater, and in 7 size fractions in river water (see Figure 4.9). Heights of the HPSEC peaks were used to quantify each fraction. The sum of peak heights (not shown) correlated well with the DOC, absorbance and aromaticity results during the river water infiltration. Based on the calibration curve, the average molecular weights for different fractions were calculated from the minimum and maximum retention times of each peak. The average molecular weights for different fractions were 2660 Da (I), 2340 Da (II), 1920 Da (III), 1410 Da (IV), 890 Da (V), 410 Da (VI) and 180 Da (VII).

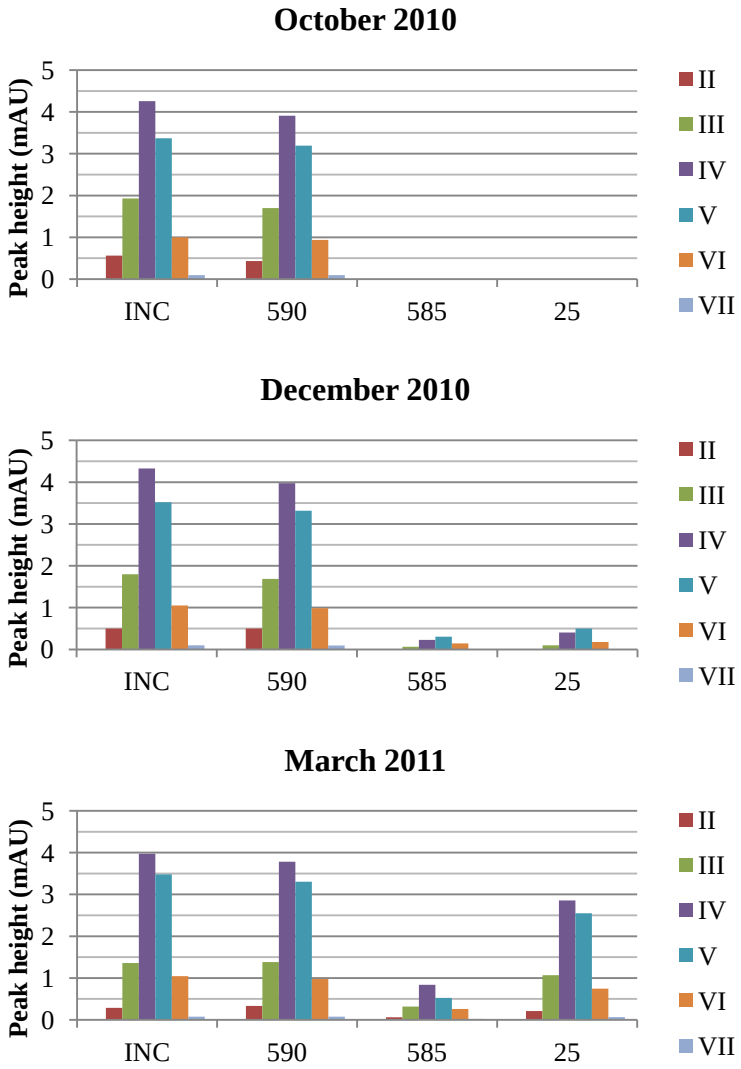

Figure 4.8. *Molecular size distribution of NOM in infiltrated water and groundwater from October 2010 to March 2011. The average molecular weights for different fractions are 2340 Da (II), 1920 Da (III), 1410 Da (IV), 890 Da (V), 410 Da (VI) and 180 Da (VII).*

In natural groundwater no fractions were detected with HPSEC in September (not shown) and October 2010. From October 2010 to March 2011 the infiltrated water and water in observation well 590 had similar molecular size distribution, where fraction IV had the highest concentration, followed by fractions V, III, VI, II and VII, respectively. River water intrusion into the aquifer was clearly evident in observation wells 585 and 25 in December 2010 and March 2011.

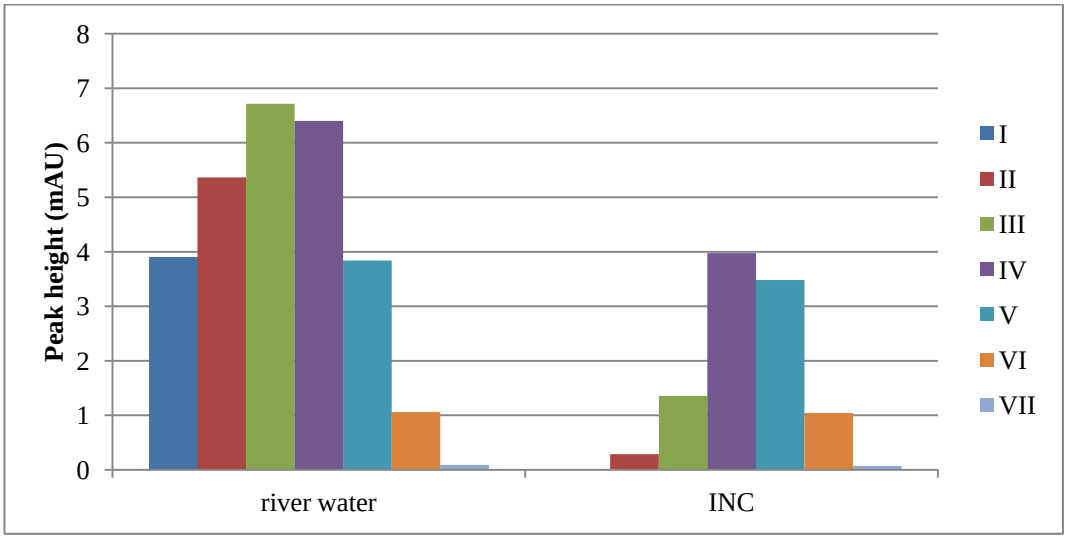

Figure 4.9. *Molecular size distribution of NOM in river water and infiltrated water in March 2011. The average molecular weights for different fractions are 2660 Da (I), 2340 Da (II), 1920 Da (III), 1410 Da (IV), 890 Da (V), 410 Da (VI) and 180 Da (VII).*

Figure 4.9 presents a comparison of the NOM molecular size distribution in river water and infiltrated water in March 2011. The results clearly show that the pretreatment significantly reduced the larger molecular fractions II, III and IV. The quantities of fractions II and III were strongly reduced by 95% and 80%, respectively, and the quantity of fraction IV was reduced by nearly 40%. The largest molecular weight fraction I was completely removed from river water. However, the pretreatment did not have a significant effect on the removal of the smaller molecular weight fractions V, VI and VII.

Figure 4.10 shows the NOM fraction compositions as peak height percentages of each fraction in December 2010 and March 2011. In December 2010, when the infiltrated water was starting to mix with the natural groundwater, the percentages of different NOM fractions in observation wells 585 and 25 differed significantly from the NOM composition of infiltrated water and observation well 590. The largest fraction II and the smallest fraction VII were not detected, and fractions V and VI appeared in higher relative concentrations compared to the NOM compositions in infiltrated water and observation well 590. In March 2011, the NOM fraction compositions in observation wells 585 and 25 were relatively similar with the NOM compositions in infiltrated water

and water in observation well 590. However, compared to the NOM compositions in other sampling locations, slightly higher relative concentrations for fractions III, IV and VI, and lower relative concentration for fraction V were measured in observation well 585.

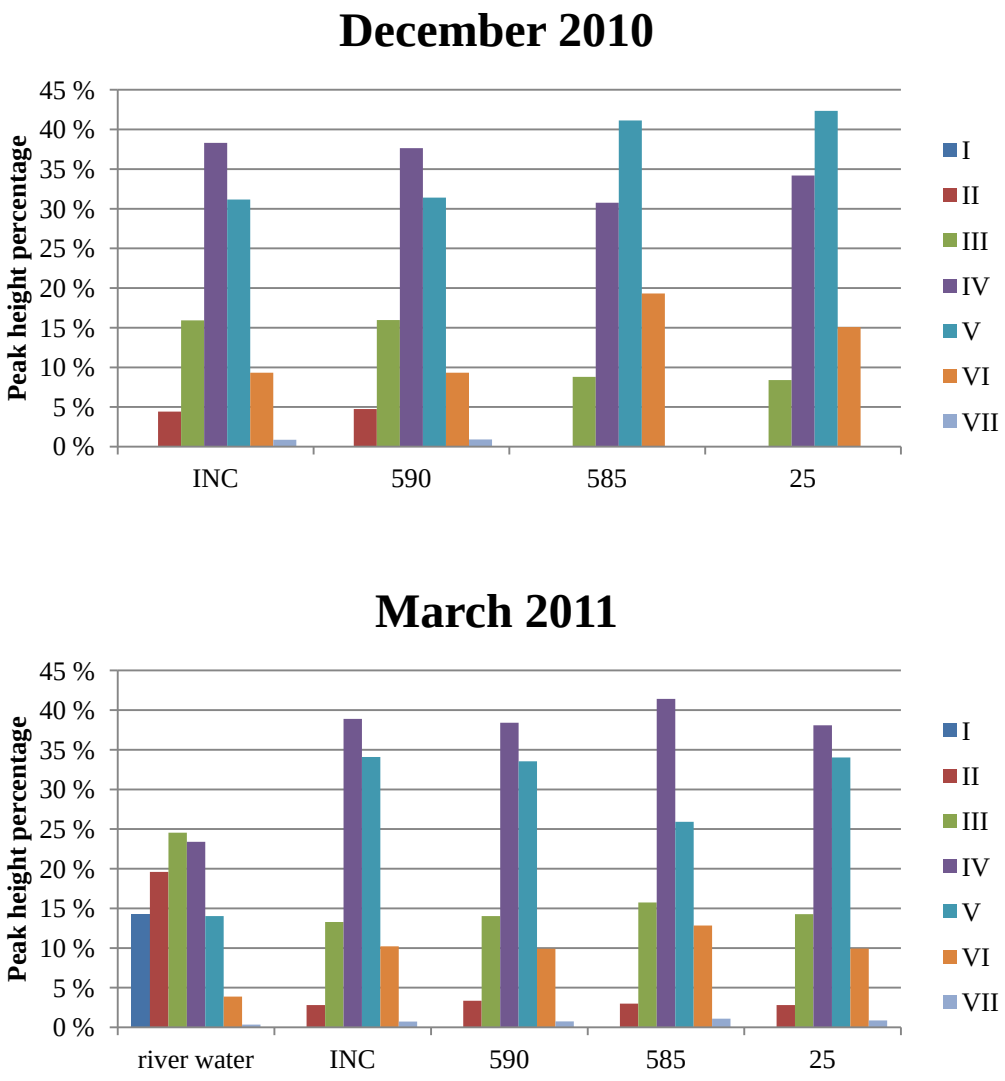

Figure 4.10. *Peak height percentages of the NOM fractions in December 2010 and March 2011. Average molecular weights of size fractions I-VII are listed in Figure 4.9.*

The results show that the pretreatment changed the river water NOM fraction composition significantly, as discussed earlier. In December 2010, when infiltrated water was intruding into the natural aquifer, the small molecular fractions V and VI drifted faster with infiltrated water than larger fractions II, III and IV. This is partly in accordance with the observations that the mobility of NOM increases with decreasing molecular weight and hydrophobicity (Tufenkji et al. 2002). However, against the observations, the smallest molecular fraction VII was not detected in observation wells

585 and 25 in December 2010. This could be explained by biodegradation of the smallest fraction, or by chemical characteristics of the fraction which may affect its mobility. In March 2011, when the system was more filled with infiltrated water, the NOM compositions in observation wells 585 and 25 had changed to fairly similar with the NOM compositions of infiltrated water and groundwater in observation well 590. The slightly differing NOM composition in well 585 may indicate that the proportion of infiltrated water had not yet stabilized, and that the water quality was still changing because infiltrating water and native groundwater were mixing. This would be in accordance with the low DOC concentration in the well.

Several studies from different MAR sites in Finland and elsewhere, and results from column experiments have demonstrated preferential removal of large molecular weight fractions during soil aquifer treatment (Kolehmainen et al. 2007; Maeng et al. 2011b). Studies concerning the use of DOM fractions by heterotrophic bacteria have yielded contradictory results (Kolehmainen et al. 2009b), and it has been shown that the characteristics (for example, fulvic-like, protein-like, and polysaccharide-like substances) of NOM fractions can vary significantly in different water samples (Her et al. 2003). The HPSEC results in this study indicated preferential removal of large molecular weight fractions between infiltration basins and observation well 590 in October 2010 (detailed data not shown). However, the results in December 2010 and March 2011 showed no preferential removal of any fraction between the infiltration ponds and observation well 590, and indicated rather the intrusion of infiltrated water into observation wells 585 and 25. Analyzing NOM for example with HPSEC-UVA-fluorescence-DOC system could give more information of the characteristic of different NOM fractions (Her et al. 2003) and help to better understand the removal of different fractions and water quality changes during MAR.

4.5. Phosphorus and nitrogen analyses

Table 4.2 presents the concentrations of total phosphorus, nitrate, nitrite and ammonium in the infiltrated water and observation wells 590, 585 and 25 in March 2011. Infiltrated water was low in phosphorus, the concentration being under the detection limit. The phosphorus concentration in the aquifer varied between 7 and 11 µg/l. The most common inorganic nitrogen compound was nitrate that varied between 170 and 560 µg/l in concentration in the aquifer. In observation well 590 nitrate and nitrite concentrations were slightly higher when compared with infiltrated water. Nitrite concentrations in observation wells 585 and 25 were under the detection limit. Ammonium concentrations were under the detection limit in the aquifer, but the concentration in the incoming water was 19 µg/l.

Table 4.2. *Nitrogen and total phosphorus in water samples in March 2011.*

Sampling location	Total P (µg/l)	NO_3^- -N (µg/l N)	NO_2^- -N (µg/l N)	NH_4^- -N (µg/l)
INC	<5	550	1,2	19
590	7	560	1,9	<7
585	11	170	<1	<7
25	8	310	<1	<7

In a preliminary study at Virttaankangas conducted by the Department of Chemistry and Bioengineering of Tampere University of Technology, the chemical composition of native groundwater samples from the actual AGR site and the immediate surroundings were analysed. Total phosphorus in the esker core aquifer was approximately 15 µg/l, concentration of both nitrate and nitrite together approximately 0,069 mg/l, and ammonium concentrations under the detection limit, less than 0,003 mg/l. Kortelainen and Karhu (2009) reported nitrate concentrations of less than 0,2 mg/l in the groundwater flow path before the eastern AGR site, and 0,71 mg/l at production well K51.

Total phosphorus results indicate, that the infiltrated water diluted phosphorus concentrations in the aquifer. In observation well 590 the concentration was higher than in infiltrated water, suggesting slight phosphorus increase from within the aquifer. Concentrations in observation well 585 and 25 were in accordance with other results indicating higher influence of infiltrated water in observation well 25 compared to observation well 585. In Finland, phosphorus has been demonstrated to limit the growth of heterotrophic bacteria in drinking water (Keinänen et al. 2002), and it was also assumed to be the limiting nutrient in Tuusula AGR site in a study by Kolehmainen et al. (2009a). Lehtola et al. (2002) reported that soil infiltration decreased phosphorus concentration to less than 2 µg/l in studied Finnish groundwater and AGR sites. However, the results from Virttaankangas do not directly indicate phosphorus utilization by bacteria.

Inorganic nitrogen results indicate that ammonium is oxidized to nitrite, and nitrite partly to nitrate between infiltration basins and observation well 590. During further groundwater flow, the rest of nitrite is oxidized to nitrate. Elevated nitrate concentrations due to influence of infiltrated water in observation wells 585 and 25 are in accordance with other results.

4.6. Total cell counts

The results of the DAPI counts in water phase samples are presented in Figure 4.11. The error bars represent the standard deviations of 40 bacterial cell counts (two replicate counts, both including 20 bacterial cell counts each).

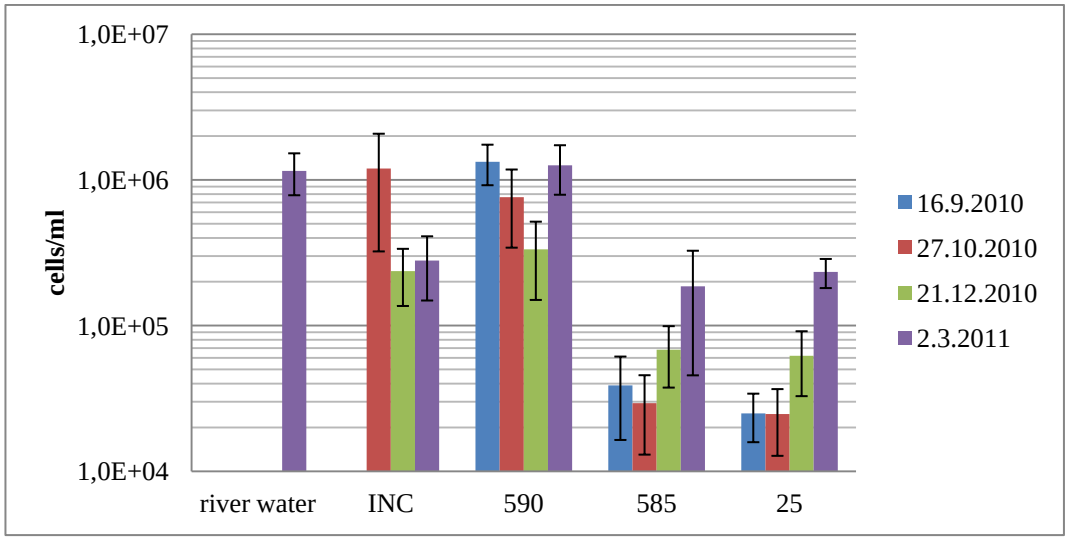

Figure 4.11. *DAPI count results of water phase samples during river water infiltration. Error bars represent the standard deviation of 40 bacterial cell counts. Note the logarithmic scale on the y-axis.*

In March 2011, the bacterial cell density in river water was $1{,}2\times10^6$ cells/ml. Pretreatment decreased the cell abundance to $2{,}8\times10^5$ cells/ml in infiltrated water. Bacterial cell abundance in infiltrated water varied between $2{,}4\times10^5$ and $1{,}2\times10^6$ cells/ml, being highest in October 2010 and relatively stable in December 2010 and March 2011.

In native groundwater (September 2010 and in observation wells 585 and 25 in October 2010), high cell abundance was observed in observation well 590 ($1{,}3\times10^6$ cells/ml) compared to the cell abundances in other observation wells ($2{,}5\times10^4$-$3{,}9\times10^4$ cells/ml). The results are in accordance with commonly observed cell numbers in natural groundwaters (10^2-10^6 cells/ml, Giebler and Lueders 2009). In native groundwater, the cell densities along the flow path on average ($2{,}9\times10^5$ cells/ml) were in agreement with the observation gained from the preliminary study at Virttaankangas ($2{,}8\times10^5$ cells/ml, on average). In observation well 590, both the significantly higher cell density and the higher aromaticity in natural groundwater indicated local differences in the water quality within the aquifer, as discussed in section 4.3.

During the sampling period, the cell counts in observation well 590 varied between $3{,}3\times10^5$ and $1{,}3\times10^6$ cells/ml. The densities were higher compared to the infiltrated water in December 2010 and March 2011, and the results show that the cell densities in

sub-surface varied with time. DAPI count results from observation wells 585 and 25 show similar trends with each other: in September and October 2010 the cell abundances were stable in both observation wells, but the river water intrusion into the aquifer was clearly evident in December 2010 and March 2011, thus correlating with the TOC/DOC, absorbance and HPSEC results. In December 2010 the cell abundance had increased to $6{,}8\times10^4$ cells/ml and to $6{,}2\times10^4$ cells/ml in observation wells 585 and 25, respectively. In March 2011 the abundances increased to the same level with the infiltrated water, being $1{,}9\times10^5$ and $2{,}3\times10^5$ cell/ml, respectively.

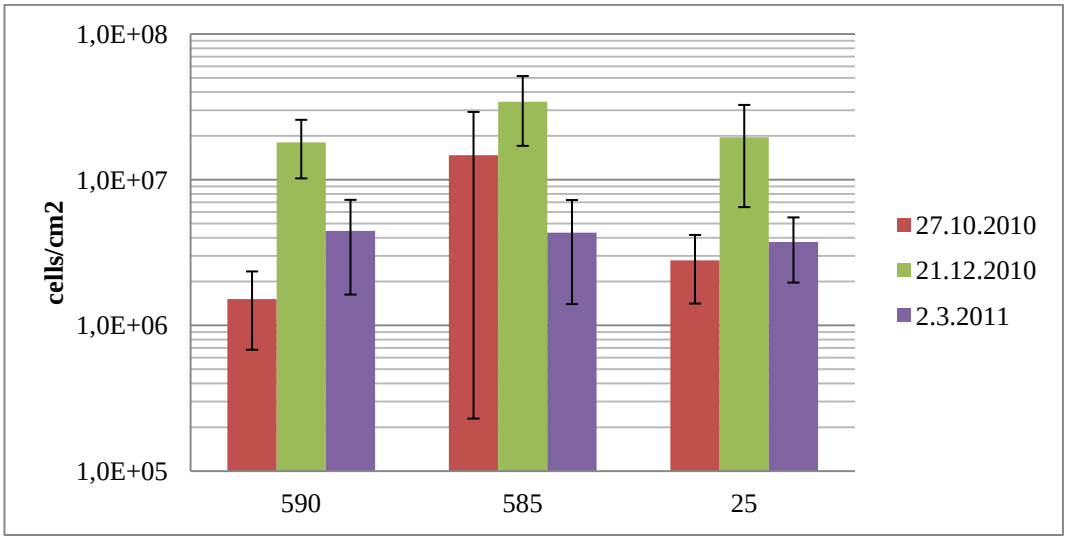

Figure 4.12. *DAPI count results of attached phase samples during river water infiltration. Error bars represent the standard deviations of 40 bacterial cell counts. Note the logarithmic scale on the y-axis.*

The results of the DAPI counts in the attached phase samples are presented in Figure 4.12. Unlike in the water phase, the attached phase DAPI count results showed slightly lower or similar bacterial cell abundances in observation well 590 in comparison to observation wells 585 and 25. The attached phase DAPI count results were dynamic and varied between $1{,}5\times10^6$ and $3{,}4\times10^7$ cells/cm^2 in all three observation wells. The highest abundances were observed in December in all observation wells, but the influence of water quality changes to cell numbers was not evident, unlike in water phase samples in observation wells 585 and 25.

In the preliminary study at Virttaankangas, the attached phase cell counts were lower on average, $5{,}6\times10^5$ cells/cm^2. The cell counts in the preliminary study were performed directly from the biofilm collector slides, which probably is the reason for the lower cell count results. According to Giebler and Lueders (2009), the total number of bacteria ranges between 10^4 and 10^8 cells/cm^3 of sediment. The attached phase results cannot be directly compared with results presented in volumetric units, but it can be deduced that the attached phase cell density is higher compared to the water phase cell density.

Kolehmainen et al. (2007; 2009b) showed that suspended bacterial cell numbers decreased along the flow path in a full scale AGR site in Tuusula and in a soil column experiment. This trend was not observed in Virttaankangas aquifer.

4.7. Bacterial community analyses

4.7.1. Water phase bacterial communities

Figures 4.13 and 4.14 present the water phase bacterial community profiles in the four samplings during the river water infiltration. The results of bacterial community analysis show the PCR product lengths of the detected bands in base pairs, and the relative quantities (%) of the bands in each sample. Each band represents different bacterium or bacteria with a certain PCR product length.

In September 2010 (Figure 4.13), the microbial community profile illustrates the bacterial communities in the native aquifer since the infiltrated water had not yet reached any of the observation wells. The community profiles in observation wells 585 and 25 were similar with each other and showed high diversity (31 and 36 peaks, respectively) of different bacteria in the aquifer. Despite of the high diversity, it should be noticed that the relative quantity of many detected bands was less than 2%. The bacterial community in observation well 590 was slightly different and less diverse (19 peaks) compared to the communities in the two other observation wells.

In October 2010 (Figure 4.13), the bacterial community in observation well 590 was affected by infiltrated water, showing similar community profile and relative quantities of the peaks with the community profile of infiltrated water. The bacterial community of infiltrated water was less diverse compared to the aquifer community. The native groundwater bacteria community was still clearly present in observation wells 585 and 25 showing that infiltrated water had not reached the end of the flow path. The community profiles in these two observation wells were similar in September and October 2010, but the relative quantities of certain bands had changed.

In December 2010 (Figure 4.14), the bacterial communities in observation wells 585 and 25 had clearly changed from the native groundwater communities. This observation is in accordance with other results in this study, indicating that infiltrated water had reached the two wells. The change was seen as lower diversity of the bacterial communities in the observation wells, and especially as changes in the relative quantities of the bands presenting different bacterium. In September and October, a slightly dominating group with PCR product length between 470-475 bp could be discovered in native groundwater, but otherwise the quantities of different aquifer bacteria were fairly even. Compared to October 2010, more diversity in region 530-545 bp could be seen in observation well 590. The profile of the infiltrated water had three

clearly dominating groups with PCR product lengths of approximately 470-475 bp, 500-505 bp and 520 -527 bp, and these groups were also clearly present in all observation wells in December 2010.

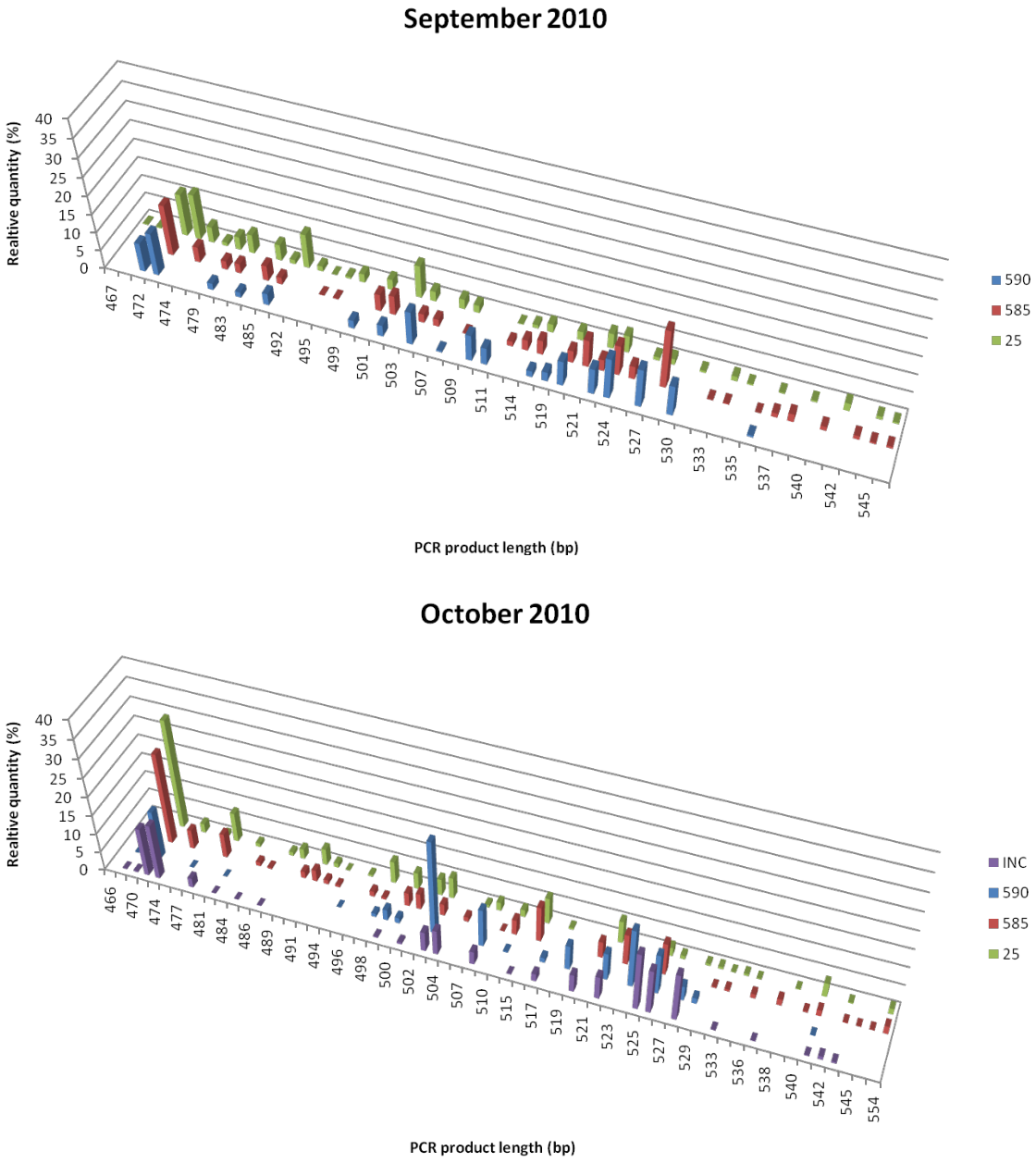

Figure 4.13. *Bacterial community profiles in water phase samples in September and October 2010. Each bar represents different bacterium/bacteria with a certain PCR product length (x-axis) and its/their relative quantity in the sample (y-axis). The lengths of the PCR products have an error of ±1 bp.*

The bacterial community profile in March 2011 (Figure 4.14) was similar to the profile of December 2010, but a shift further towards the bacterial community of the infiltrated water could be seen in observation wells 585 and 25. The results also show that

pretreatment of river water slightly changed the community profile of infiltrated water, but the dominating groups in river water and pretreated water remained the same. The results indicate that the bacterial communities in all samples stayed fairly stable between the last two samplings. Additionally, the dominating groups remained the same along the groundwater flow path, which is contrary to the observations by Kolehmainen et al. (2007; 2008), who showed changes within bacterial community structures during infiltration in three Finnish AGR sites and in a pilot-scale sand column experiment.

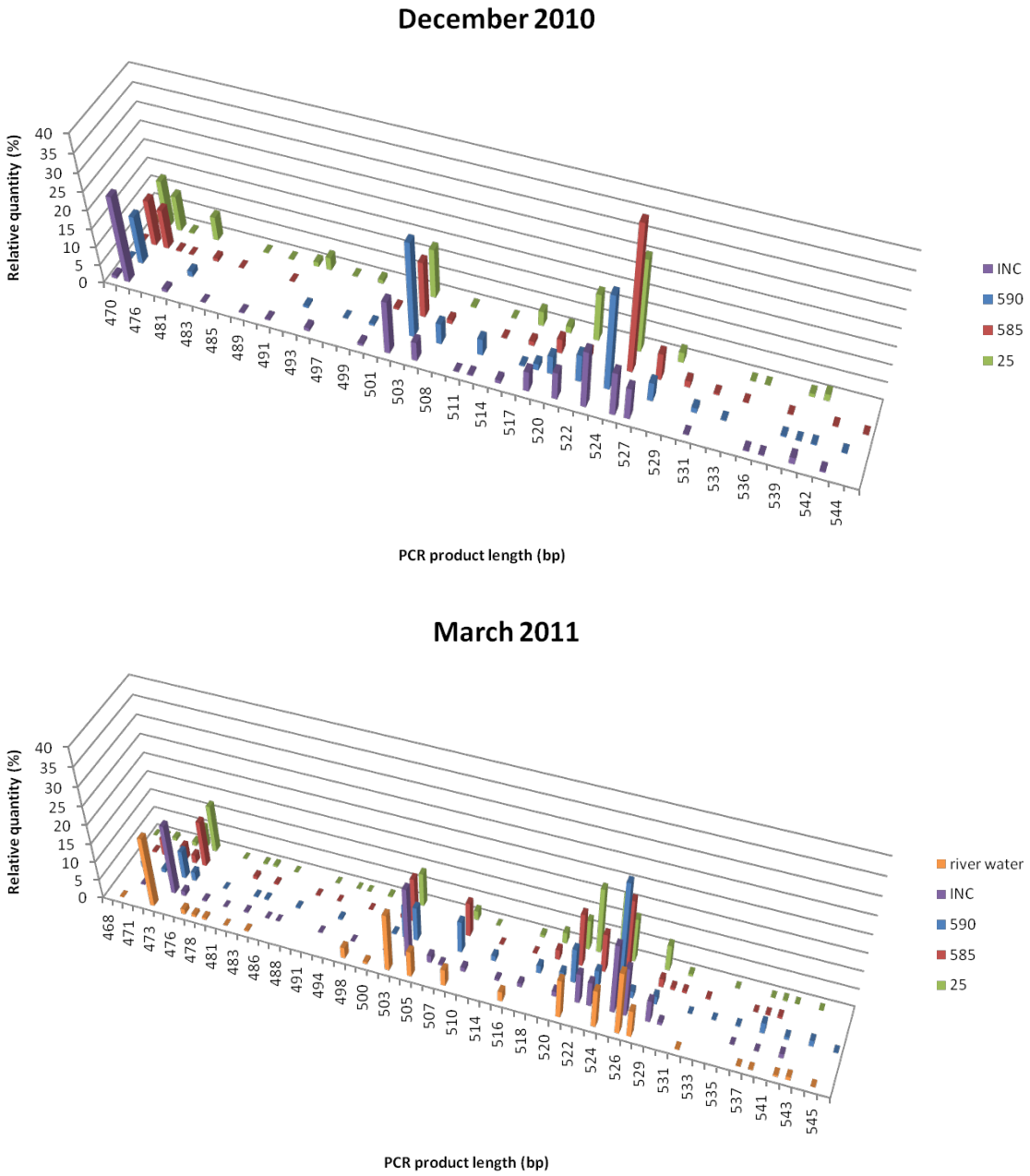

Figure 4.14. *Bacterial community profiles in water phase samples in December 2010 and March 2011. Each bar represents different bacterium/bacteria with a certain PCR product length (x-axis) and its/their relative quantity in the sample (y-axis). The lengths of the PCR products have an error of ±1 bp.*

Figure 4.15 presents all water phase community profiles to enable comparison between the profiles during the river water infiltration period. For the infiltrated water and the water samples from observation wells 590, 585 and 25, the diagram shows the profile of the first sampling in foreground, followed by the profiles of later samplings.

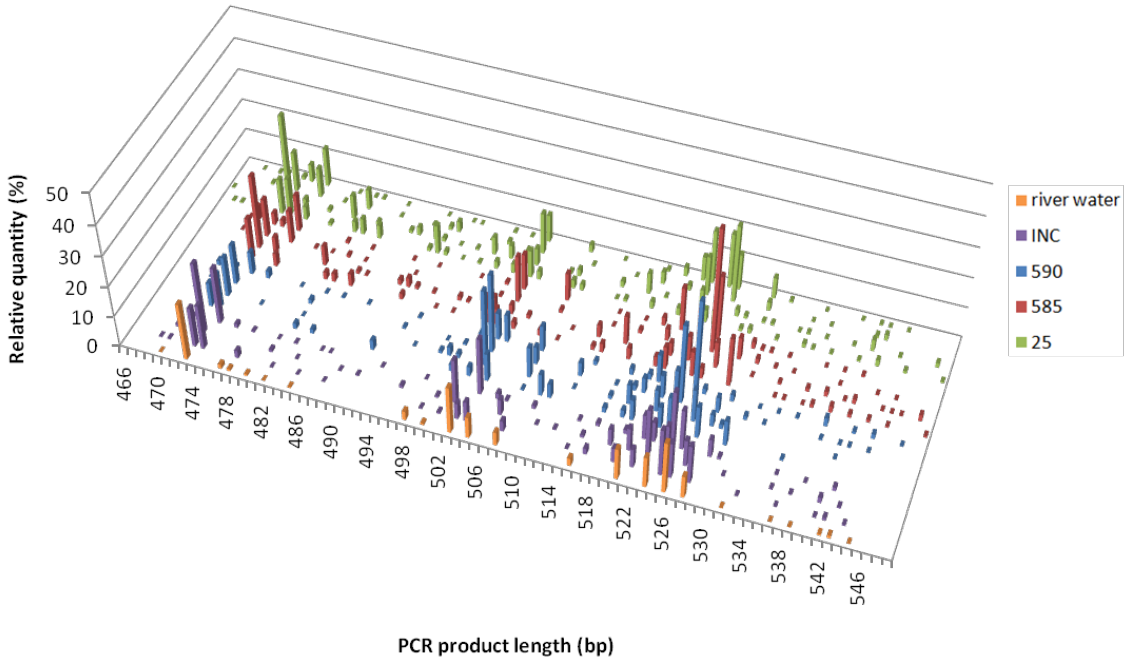

Figure 4.15. *Comparison of the water phase bacterial community profiles in river water, infiltrated water and observation wells 590, 585 and 25 during the sampling period. The profiles for infiltrated water and groundwater samples are presented in the sampling order beginning from the profile of the first sampling.*

The bacterial community in infiltrated water remained fairly stable during the sampling period. In the second sampling, a shift towards the infiltrated water profile can be seen in observation well 590 as a change in the community profile, and as increased community diversity during the last two samplings in PCR product length region 530-546 bp. Likewise, a change from the native groundwater community profile towards the infiltrated water profile is clearly observed in observation wells 585 and 25. Infiltrated water and AGR-affected community profiles had 8 to 10 clearly dominating peaks. Native aquifer community profiles had 10-12 peaks which have relative quantity of over 3%. Kolehmainen et al. (2007) reported similar results showing that AGR-affected community profiles differed clearly from the natural groundwater community profiles.

For more detailed analysis of the native groundwater bacterial community a clone library was constructed from the LH-PCR product from observation well 585 (September 2010). Most of the sequenced bands had closest relatives in uncultured bacteria which were mostly phylogenetically unknown. The sequences between 470 and

480 bp belonged mainly to α-Proteobacteria, sequences between 500 and 504 bp to Sirochaetes and Actinobacteria, and sequences between 522 and 524 bp to Proteobacteria. When the sequences were compared to closest cultured bacteria, the results indicated that the bacterial groups with PCR product lengths around 520-530 bp belonged to β- and γ-Proteobacteria and Bacteriodetes, sequence around 538 bp to Nitrospirae and sequence around 550 bp to δ-Proteobacteria.

The AGR-affected groundwater community profile had three clearly dominating groups with PCR product lengths of 470-475 bp, 500-505 bp and 520 -527 bp. The sequencing results indicate that these lengths correspond to bacteria belonging to α-Proteobacteria, Sirochaetes and Actinobacteria, and β- and γ-Proteobacteria and Bacteriodetes, respectively. Likewise, in the beginning of a pilot-scale sand column experiment by Kolehmainen et al. (2008), α-, β- and γ-Proteobacteria and Actinobacteria were identified as dominant groups in water samples from the sand column. Compared to the bacterial community in infiltrated water, the changes in bacterial community along the flow path were not as significant in the beginning of the experiment as they were after the system had stabilized almost after 2 years of operation. In the stabilized system, α- and δ-Proteobacteria and Verrucomicrobia were identified as dominant groups. Kolehmainen et al. (2007; 2008), among others, have demonstrated community structure changes during soil-aquifer treatment, and the column experiment results could indicate that, with time, the water phase bacterial community profile may further develop along the flow path also in Virttaankangas.

4.7.2. Attached phase bacterial communities

Figure 4.16 shows the attached bacterial community profiles in observation wells 590, 585 and 25 during the river water infiltration. The community profiles from observation wells 585 and 25 in October 2010 demonstrate the native aquifer attached phase community. The community diversity and quantities of the peaks varied slightly between the sampling locations and samplings, but no considerable changes could be detected in the profiles during river water infiltration. Therefore the influence of infiltrated water on attached phase communities was not evident. Highest community diversity was observed in March 2011, but the low relative quantity of many detected bands should be noted. In observation well 25, a more apparent shift of dominating groups was seen in March 2011. Changes of the dominating peaks were more dynamic in observation well 585 compared to the other observation wells.

For the attached phase, a clone library was constructed from the LH-PCR product from observation well 25 (March 2011). The sequenced bands had closest relatives in uncultured bacterium clones, mostly phylogenetically unknown. When the sequences were compared to closest cultured bacteria, the results indicated that the sequence with PCR product length 514 bp belonged to γ-Proteobacteria, and the sequences from 521 to 524 bp to β-Proteobacteria.

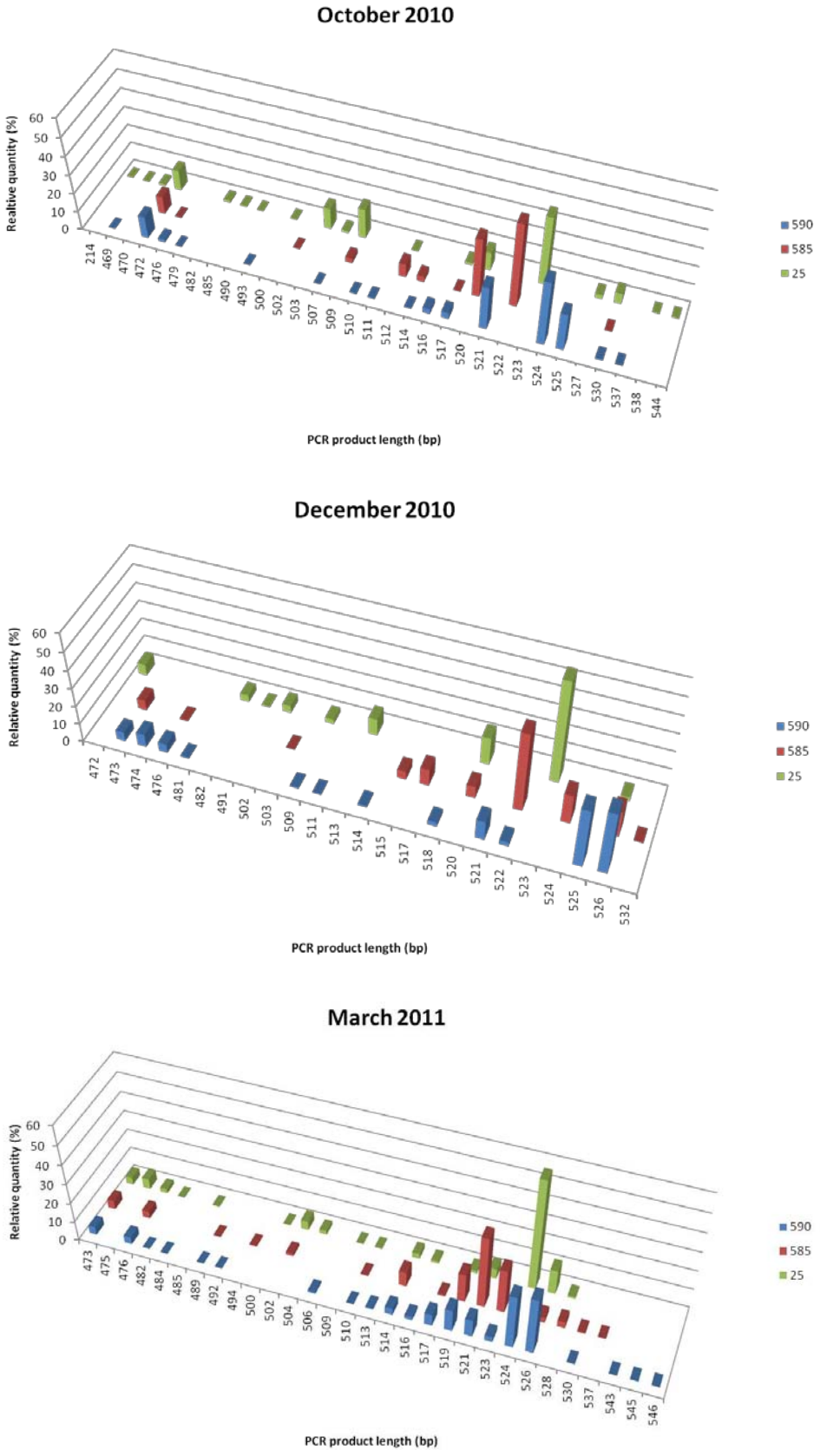

Figure 4.16. *Attached bacterial community profiles during the sampling period. Each bar represents different bacterium/bacteria with a certain PCR product length (x-axis) and its/their relative quantity in the sample (y-axis). The lengths of the PCR products have an error of ±1 bp.*

Figure 4.17 presents a comparison of the attached bacterial community profiles from all observation wells during the river water infiltration period. The profiles for each observation well are presented in the sampling order so that the profile from the first sampling is presented in the foreground. It can be concluded that the dominating groups and their relative quantities stayed fairly stable during the whole sampling period. Attached bacterial communities had 3 to 6 dominating peaks. In each observation well, the dominating groups had peaks with PCR product lengths of approximately 520-527 bp, corresponding to β-Proteobacteria. However, the dominant peaks were slightly shifted between the three observation wells. Kolehmainen et al. (2008) reported that several Proteobacterial groups were dominant on carrier particles in a fluidized-bed reactor simulating AGR. However, in AGR simulations, the most dominant subclasses of sessile Proteobacteria have been reported to be γ-, β- and α-Proteobacteria, in this order (Kolehmainen et al. 2008).

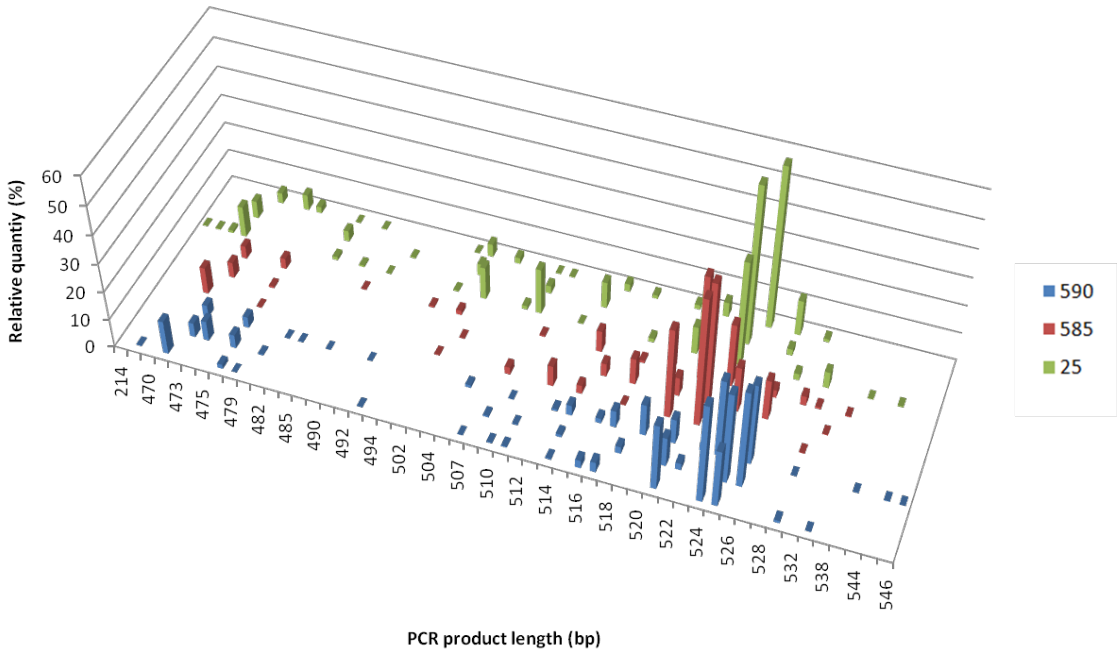

Figure 4.17. *Comparison of the attached bacterial community profiles in different observation wells during the sampling period. The profiles for each observation well are presented in the sampling order beginning from the profile of the first sampling.*

A comparison between the water phase and attached bacterial community profiles is presented in Figure 4.18. Attached bacterial communities had one clearly dominating group in region 520-527 bp and another group present in each observation well with PCR product lengths of 472-476 bp. In comparison, water phase communities had three dominating groups with PCR products of approximately 470-475 bp, 500-507 bp and 520-530 bp in length.

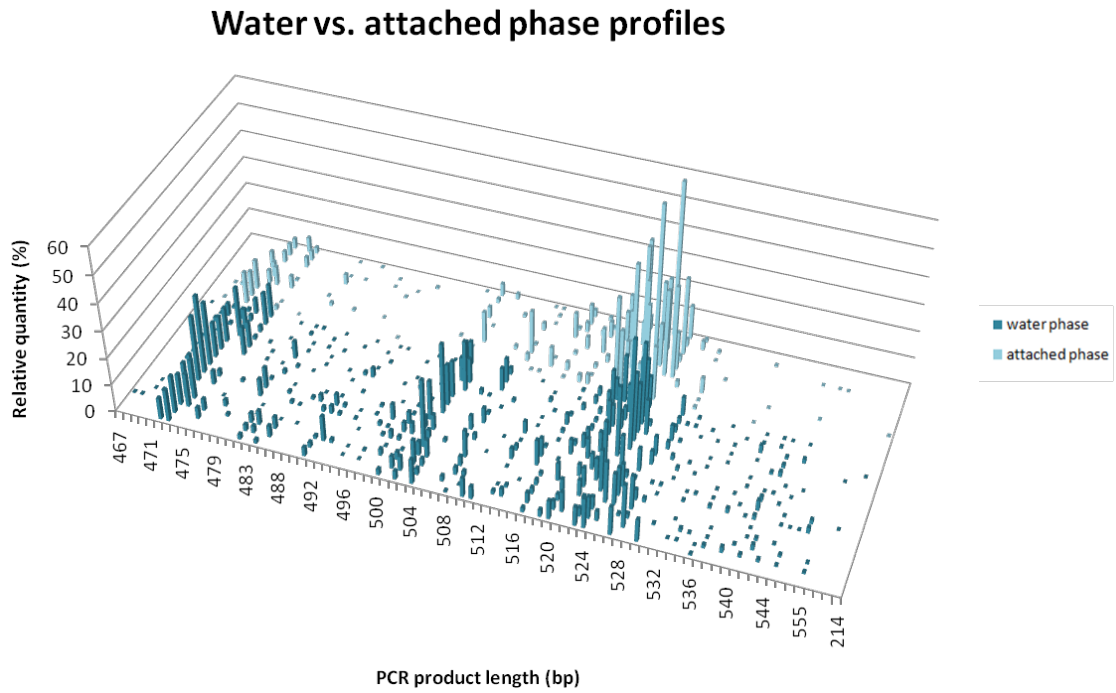

Figure 4.18. *Comparison of the water phase and attached bacterial community profiles at Virttaankangas during river water infiltration.*

Figure 4.18 shows clearly that the water phase bacterial communities were more diverse compared to the attached bacterial communities. This is in contrary to observations of Kolehmainen et al. (2008), who reported more diverse communities in biofilms compared to unattached communities in experimental AGR. Additionally, the attached and unattached communities differed greatly. However, in Virttaankangas, the two dominant peaks in biofilm communities (472-474 bp and 520-526 bp) were also detected in water phase communities, and other similarities between the water and attached phase communities could also be detected.

4.7.3. Soil bacterial communities

The characterisation of soil bacteria communities was performed in order to evaluate the feasibility of the HDPE biofilm collector slides for aquifer biofilm sampling by comparing the attached bacterial communities in soil samples and on biofilm collectors with each other. Table 4.3 lists the sample types (soil or biofilm collector), sampling depths and the soil fractions at the sampling depths for each sample. The depths of the soil samples were measured from the ground surface level and the depths of the biofilm collectors from the top of the observation well. The top of the observation wells were approximately 1 m above the ground surface level.

Table 4.3. *Soil fractions in different sampling depths. Soil sample depths are measured from the ground surface level and biofilm collector depths from the top of observation wells.*

Well	Depth (m)	Sample type	Soil fraction
590	19-20	soil sample	gravel
	25	biofilm collector	gravel
	27,5-28,5	soil sample	gravel
585	18,5-19,5	soil sample	sandy gravel
	28-29	soil sample	gravel
	35	biofilm collector	gravel
	38,5-39,5	soil sample	gravel
25	18-19	soil sample	gravelly sand
	25	biofilm collector	stony sand
	35-36	soil sample	not defined

Figure 4.19 shows the comparison of attached bacterial community profile and soil bacterial community profiles in each observation well. Results of the attached phase community profiles are taken from the first attached phase sampling in October 2010. The soil samples from different depths were collected from the observation well locations before the start of the river water infiltration, in connection of well-drillings.

In observation well 590 the soil samples had fairly similar community profiles. The sample from 19-20 m depth showed slightly higher diversity. Attached bacterial community had more diversity compared to the soil samples but the dominating peaks of attached community had similar PCR product lengths as the peaks in the soil samples.

In observation well 585 all the soil bacterial communities from different depths differed from each other. The sample from 18,5-19,5 m depth had dominating bacterial groups in the same PCR product length region (520-525 bp) as the soil sample from 28-29 m depth but showed the highest diversity. The soil sample community profile from 38,5-39,5 m depth differed clearly from the two other soil community profiles by having two dominating groups in PCR product lengths of approximately 510 and 518 bp. The attached bacterial community in observation well 585 had dominating groups in PCR product lengths of approximately 510 and 520-522 bp and the attached community profile seemed to have influences from the both soil communities which are found in 28-29 m and 38,5-39,5 m depths.

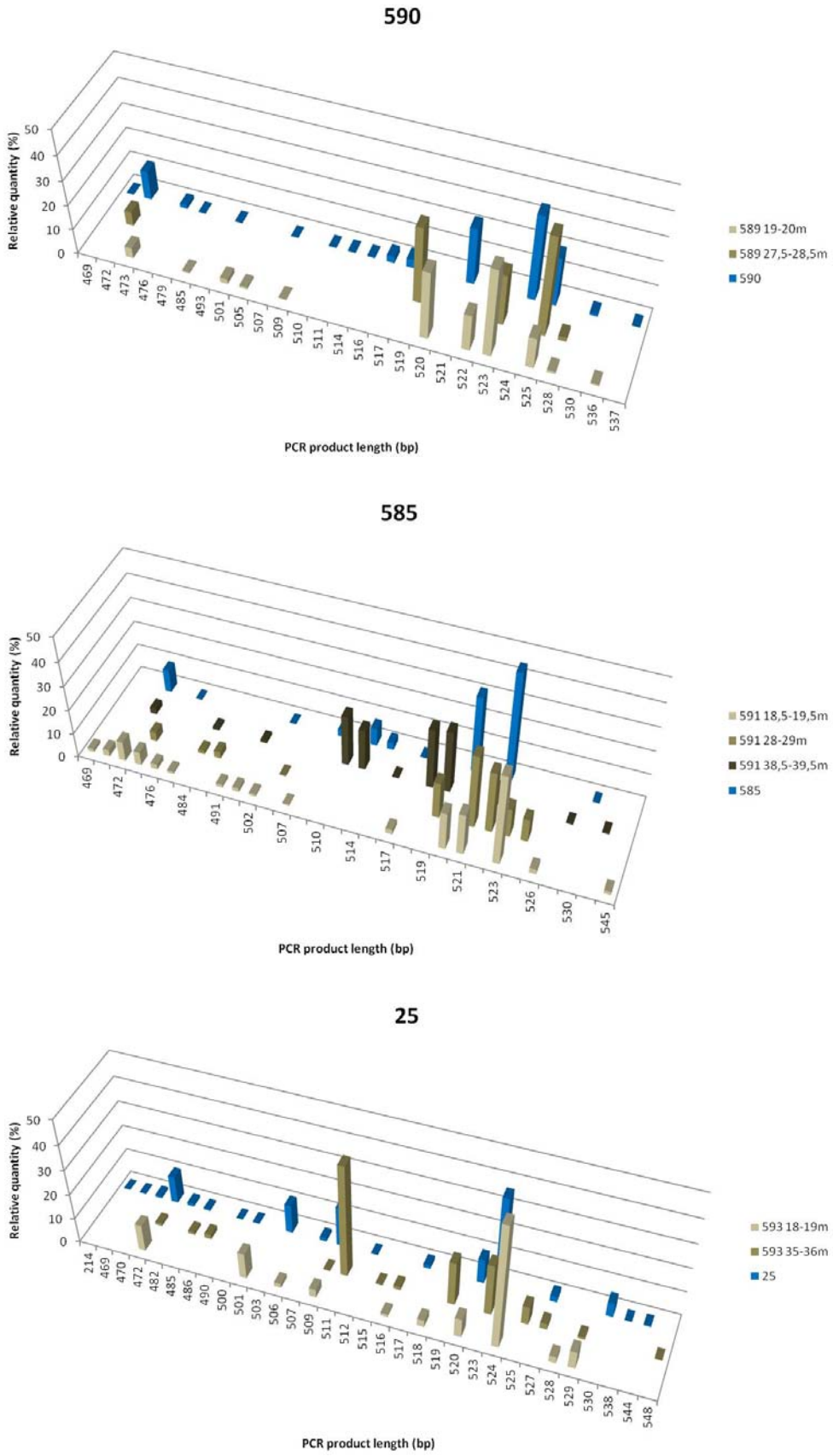

Figure 4.19. *Comparison of the bacterial community profiles in attached phase samples (blue) and soil samples in each observation well. Each bar represents different bacterium/bacteria with a certain PCR product length (x-axis) and its/their relative quantity in the sample (y-axis). The lengths of the PCR products have an error of ±1 bp.*

In observation well 25 the soil sample bacterial communities in 18-19 m and 35-36 m depths differed significantly from each other when it comes to the relative quantities of the dominating peaks and their PCR product lengths. In 18-19 m depth the dominating peaks had PCR product lengths of 472, 501, 520, 524 and 529 bp whereas in the depth of 35-36 m the PCR product lengths of the dominating groups were 511, 519 and 523 bp. The diagram shows that the attached bacteria community profile was similar to the soil bacteria community profile in 18-19 m depth, which is closer to the biofilm collector when compared to the soil sample in 35-36 m depth. However, the attached community profile showed a higher diversity.

Considering the error of ±1 bp, the results show that the dominating bacterial groups in soil samples were also present in the attached bacterial communities at similar quantities in observation well 590 and 25. The biofilm collector in observation well 585 had peaks at similar PCR product lengths with the soil samples, but the relative quantities were not as representative as in the two other observation wells. The results indicate that the HDPE biofilm collector slides can be representative as attached phase samplers. However, biofilm collectors had higher microbial diversity compared to the soil samples in two observation wells.

According to the results, the bacterial community composition can significantly differ within the same soil fraction in different depths. This indicates that the composition in soil depends mostly on the depth of the sample. Soil fractions containing sand seem to have higher diversity of bacteria compared with soil fractions consisting only of gravel. The observations are in accordance with a number of studies, in which bacterial community composition changes with soil depth, also within same soil texture, has been demonstrated. In the sub-surface, differentiation of soil microbial communities may be caused by a number of factors, including local hydrogeochemistry, resource availability and quality, dispersal of microbes from overlying unsaturated zones, and the simple food-web structure in aquifers, among others. (Fierer et al. 2003; Giebler and Lueders 2009)

4.8. RNA assay

Figure 4.20 shows the DNA- and RNA-based bacterial community profiles of all water phase samples in March 2011. It can be seen that the DNA and RNA profiles differed significantly. All of the water phase RNA profiles were lower in diversity when compared to the DNA profiles, and had clearly one strongly dominating peak with PCR product length of 529 bp. Surprisingly, the RNA peak at the 529 bp length was very strong in all observation wells having relative quantities between 71% and 93% whereas the equivalent DNA peaks were small having relative quantities between 2% and 7%. In the river water and infiltrated water the dominating RNA peak at 529 bp had a lower relative quantity (37-40%), and the RNA profiles had several other strong peaks. The

RNA profiles from river water, infiltrated water and, observation well 590 showed slightly higher diversity compared to the RNA profiles of observation wells 585 and 25. Considering the error of ±1 bp in all results, the water phase RNA profile peaks matched with the DNA profile peaks. Although the relative quantities of the bands in RNA profiles were mostly small, the RNA-peaks clustered at same PCR product lengths with the dominating groups of the DNA profiles (470-475 bp, 500-507 bp and 520-530 bp).

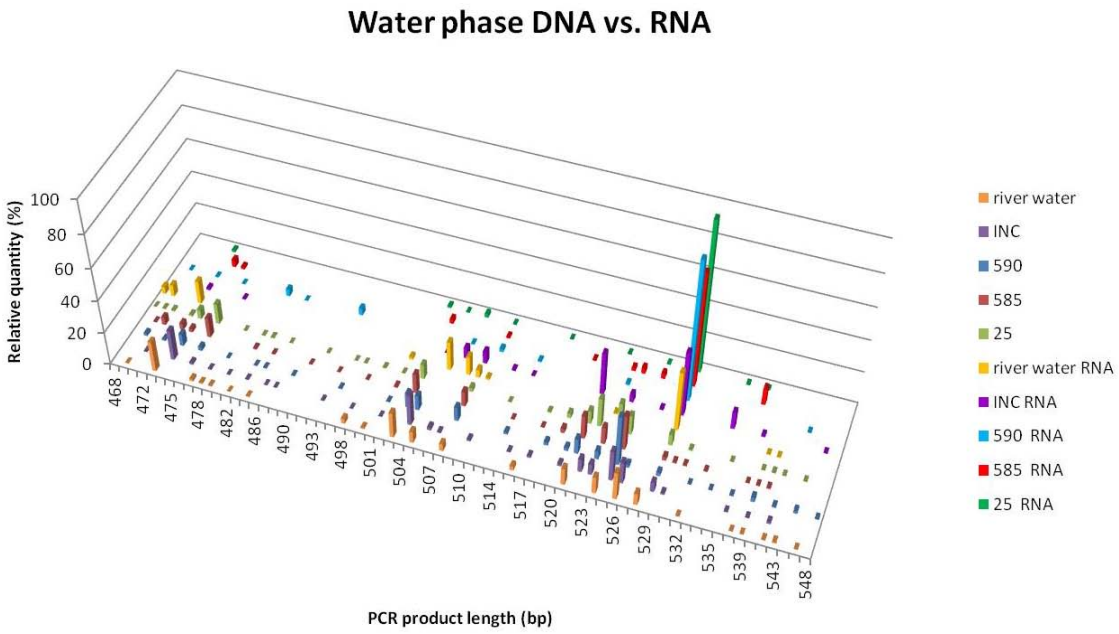

Figure 4.20. *Comparison of the DNA- and RNA-based water phase bacterial community profiles at Virttaankangas during river water infiltration on 2.3.2011. The lengths of the PCR products have an error of ±1 bp.*

Figure 4.21 shows the DNA and RNA profiles of attached bacterial communities. Again, the RNA profiles had a dominating peak on PCR product length of 529 bp and they showed less diversity compared to the DNA profiles. All three RNA profiles were fairly similar, showing weak peaks at similar PCR product lengths with the strongest DNA profile peaks. The attached phase RNA profiles had few bands which were not detected in the DNA-based community profiles.

Similarly to this study, Eichler et al. (2006) detected significant differences between the major bacterial groups in DNA- and RNA-based fingerprints from different parts of a drinking water supply system. In the study, the RNA-based fingerprints also showed bands which were not detected in DNA-based fingerprints. By comparing DNA- and RNA-based community profiles it is possible to determine the active members of the microbial communities from the members that are only present in the samples. This is based on the concept that the number of ribosomes per cell is a good measure of the

overall activity of the cell. Detection of bacterial groups only by RNA-based fingerprints was assumed to result from a high ratio of RNA to DNA in bacterial cells. A high number of ribosomes at a rather low growth rate has been demonstrated for nitrifying bacteria. (Eichler at al. 2006)

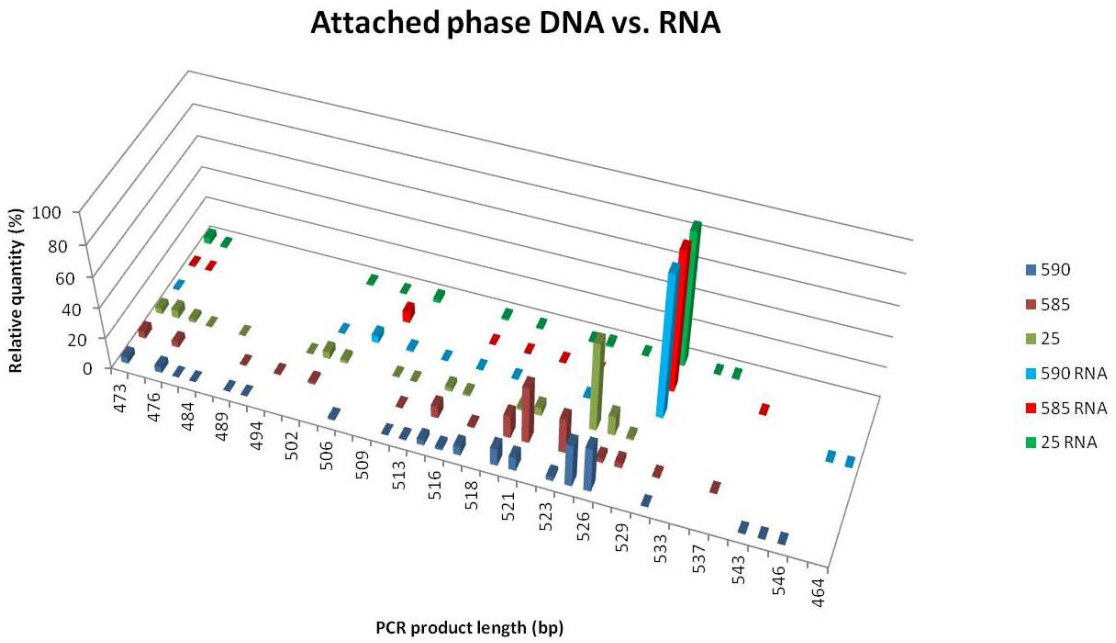

Figure 4.21. *Comparison of the attached bacteria DNA and RNA community profiles at Virttaankangas during river water infiltration on 2.3.2011. The lengths of the PCR products have an error of ±1 bp.*

The comparison of water phase and attached bacteria RNA profiles (Figure 4.22) again shows that the peak with PCR product length of 529 bp was dominant in both sample types. The relative quantity of the dominant RNA peak was 37-40% in river and infiltrated water, 71-93% in groundwater and 87-93% in biofilms. This indicates that only a few bacteria in the communities showed high activity in the aquifer. The DNA peaks with corresponding PCR product length accounted only for 0,1-7% of the whole community composition in the aquifer samples.

The diversity difference between water phase and attached phase bacterial communities is not as evident in the RNA profiles as it was in the DNA profiles (see Figure 4.18). This indicates that many unattached bacteria are inactive in the aquifer. Unattached bacteria corresponding to the dominating DNA peaks showed only minor activity. The results also demonstrate that pretreatment and especially infiltration change the functioning of the unattached bacterial community.

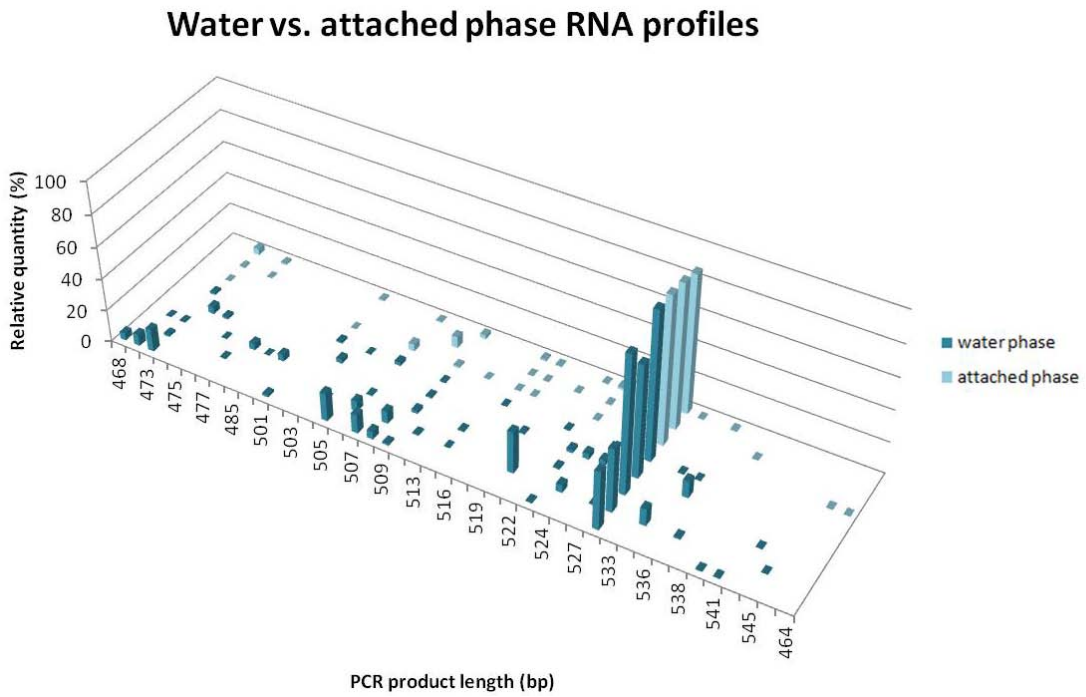

Figure 4.22. *Comparison of the RNA-based water phase and attached bacterial community profiles at Virttaankangas in March 2011.*

The attached phase RNA profile shows more similarities with the attached phase DNA profile than the comparison of water phase RNA and DNA profiles. Therefore, contrary to the activity of the water phase community, the results indicate that many of the detected attached bacteria were active in the aquifer. However, most of the attached bacteria also showed only minor activity.

The RNA results show that the DNA community profiles do not necessarily represent the functional bacterial groups in the samples. Monitoring of RNA-based bacterial community compositions could therefore be more advantageous in discovering and understanding changes in bacterial communities and their connections to the water quality in the aquifer.

4.9. Activity assay

Table 4.4 presents the activity assay results for water phase and attached phase samples in March 2011. The results include the average activities, total cell numbers in 10 ml water samples or in biofilm collectors, and the leucine uptake rates per hour per cell, calculated with Equation 3.6. For the average activities, the mean of the background counts was subtracted from the means of the sample counts. Total cell numbers were obtained using DAPI-count results from the same sampling. The average activity for the attached phase samples from observation well 590 resulted in a negative value. This means that the mean of the background counts for the controls was slightly higher compared to the means of the sample counts. Similar activities were measured from the

scintillation cocktail, the biofilm controls from every observation well, and the attached phase samples from observation well 590. This means that no or only minor leucine incorporation took place in the attached phase samples from observation well 590, and, thus, the average activity is regarded as 0 cpm.

Table 4.4. *The activity assay results for water phase and attached phase samples on 2.3.2011.*

Water phase Sampling location	**Average activity (cpm)**	**Total cells in 10 ml**	**Leucine uptake rate (mmol/cell/h)**
INC	47	$2{,}79\times10^{6}$	$6{,}85\times10^{-18}$
590	0,33	$1{,}26\times10^{7}$	$1{,}07\times10^{-20}$
585	82	$1{,}86\times10^{6}$	$1{,}79\times10^{-17}$
25	165	$2{,}34\times10^{6}$	$2{,}85\times10^{-17}$
Attached phase Sampling location	**Average activity (cpm)**	**Total cells in biofilm collector**	**Leucine uptake rate (mmol/cell/h)**
590	0	$3{,}74\times10^{7}$	0
585	2799	$3{,}64\times10^{7}$	$3{,}11\times10^{-17}$
25	9182	$3{,}15\times10^{7}$	$1{,}18\times10^{-16}$

Figure 4.23 presents the leucine uptake rates in the water phase and in the attached phase along the groundwater flow path in Virttaankangas artificial recharge site. The error bars represent the standard deviation of three replicate measurements.

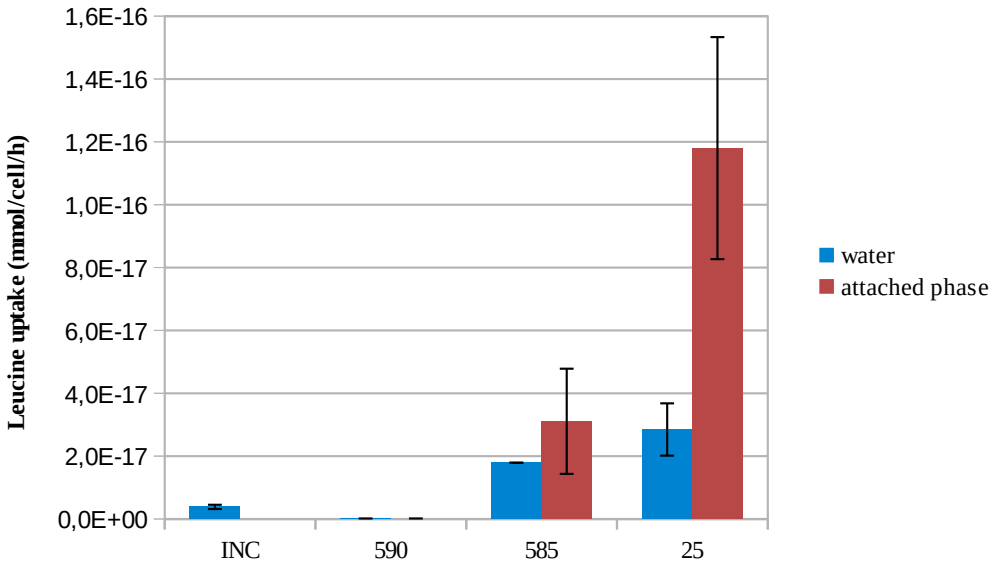

Figure 4.23. *Leucine uptake rates in the water phase and in the attached phase at Virttaankangas AGR site on 2.3.2011. Error bars represent the standard deviation of the uptake rates.*

In observation well 590, the activity in both the attached and water phase was very low, or virtually non-existent. The activity increased along the hypothetical ground water flow path in both phases. At the end of the ground water flow path, in observation well 25, the leucine uptake rates were highest both in water and attached phases, being $2{,}85\times10^{-17}$ mmol/cell/h and $1{,}18\times10^{-16}$ mmol/cell/h, respectively. The uptake rate was significantly, over four-fold higher in the attached phase compared to the water phase. Likewise, in observation well 585 the activity in the attached phase was higher compared to the water sample. However, taking the standard deviation of the attached phase uptake rate into account, the difference was less substantial. The leucine uptake rate in infiltrated water was $6{,}85\times10^{-18}$ mmol/cell/h, and thus clearly lower in comparison to the activity of the water phase cells in observation wells 585 and 25. The standard deviations were higher in the attached phase samples compared to the water phase samples.

Reason for the low microbial activity in the infiltrated water and observation well 590 is probably the low water temperature. In winter, the river water and the infiltrated water temperature is low. Additionally, in shallow infiltration basins the water temperature decreases close to 0°C and this affects strongly the temperature in observation well 590. Several studies have demonstrated that lower temperatures result in reduced biodegradation and microbial activity in MAR (Miettinen et al. 1996; Massman et al. 2006 Kolehmainen et al. 2009b).

However, temperature does not explain the differences in activity in observation wells 585 and 25. According to DOC and HPSEC results, water in observation well 25 contained mode dissolved organic carbon (3,4 mg/l) in comparison to observation well 585 (1,2mg/l) at the time of the activity sampling. The conditions in observation well 25 might therefore have been more suitable for the microbes. The HPSEC results also showed that the NOM fraction composition differed in the two observation wells. Differences in NOM quality may have an influence on bacterial activity (Miettinen et al. 1996; Schütz et al. 2010).

The higher activity of the attached bacteria indicates that the attached communities have a higher biodegradation potential than the unattached communities. This is in agreement with the theory that the attached mode of life is advantageous in an aquifer, because sediment surfaces are geochemically more diverse and offer more ecological niches than groundwater (Giebler and Lueders 2009).

5. CONCLUSIONS

Start-up experiment of a new AGR plant offered a unique possibility to investigate changes in a pristine aquifer when pretreated river water was introduced into the aquifer for the first time. Chemical and bacterial community changes were monitored along the groundwater flow path at Virttaankangas AGR site during a period of over five months. The water quality of infiltrated water differed significantly from native groundwater by having higher concentrations and different characteristics of DOC, lower pH and different bacterial community composition. Based on the monitoring results, the following conclusions were drawn:

- On-site measurements showed that calcite in Virttaankangas esker sediments increased the pH of infiltrated water, and that groundwater temperature was strongly affected by infiltrated water in the beginning of the flow path.

- The progress of the infiltrated water in the aquifer was seen as increasing DOC concentrations. Along the groundwater flow path, DOC concentrations decreased from the initial concentration in the beginning of the flow path. Within the framework of this study, the DOC removal mechanisms could not be confirmed.

- The DOC results indicated long retention time at the AGR site. The difference in DOC concentrations between observation wells in the middle (1,2 mg/l) and at the end (3,4 mg/l) of the flow path indicated that different flow paths exist in the Virttaankangas esker.

- No preferential removal of any molecular fraction of NOM occurred. In the aquifer, smaller NOM fractions moved faster with infiltrated water than larger NOM fractions.

- Infiltrated water increased the water phase cell counts in the middle and in the end of the groundwater flow path. Infiltration did not affect the attached phase cell counts.

- The bacterial community composition of native groundwater was strongly affected by infiltrated water. The progress of infiltrated water in the sub-surface was seen as decreased bacterial diversity and as a change of the natural groundwater community towards the community composition of infiltrated water. The attached bacterial communities were different from the water phase communities, and the water change did not affect the attached phase community compositions.

- RNA-based community profiles indicated that only a minor part of the attached and unattached communities showed activity in the aquifer. The LH-PCR fragment of the highly active microbial group was the same for the unattached and the attached bacteria.

- The leucine incorporation assay confirmed bacterial activity in sub-surface. Biofilms showed higher activity compared to unattached bacteria, and therefore higher biodegradation potential. Temperature affected strongly microbial activity in the infiltrated water and in the beginning of the flow path.

A longer monitoring time would have been necessary to confirm if the aquifer was still in the process of getting filled with the infiltrated water. Other methods, for instance isotope methods, are needed to differentiate the role biodegradation, adsorption and dilution in sub-surface NOM removal. RNA-based analysis was a useful way to identify the active members of the microbial communities. The method showed its potential for identifying the bacterial groups responsible of biodegradation and for better understanding the microbial responses and effects on water quality.

The dominating bacterial groups in soil accumulated on the biofilm collector slides used for sampling of the attached bacteria. However, the biofilm collectors showed higher bacterial diversity compared to those detected in soil samples. All minor bacterial groups present in soil were not detected on the slides. Therefore, the biofilm collector slides reliably collect representative bacteria that dominate in the soil matrix.

REFERENCES

Anders, R., and Chrysikopoulos, C.V. 2005. Virus fate and transport during artificial recharge with recycled water. Water Resources Research, 41, W10415.

Artimo, A., Puurunen, O., Saraperä, S. ja Ylander, I. 2007. Geologinen informaatio tekopohjavesihankkeen toteuttamisessa. Pohjavesitutkimukset Virttaankankaalla. Turku, Turun Seudun Vesi Oy. 98 p.

Bastviken, D., Olsson, M. and Tranvik, L. 2003. Simultaneous Measurements of Organic Carbon Mineralization and Bacterial Production in Oxic and Anoxic Lake Sediments. Microbial Ecology 46, pp. 73–82.

Bell, R.T. 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In: Kemp, P.F., Sherr, B.F. and Sherr, E.B. (eds.). Handbook of Methods in Aquatic Microbial Ecology. pp. 509-517.

Bournea, D.G., Blakeley, R.L., Riddles, P. and Jones, G.J. 2006. Biodegradation of the cyanobacterial toxin microcystin LR in natural water and biologically active slow sand filters. Water research 40, pp. 1294-1302.

Bouwer, H. 2000. Integrated water management: emerging issues and challenges. Agricultural Water Management 45, pp. 217-228.

Bouwer, H. 2002. Artificial recharge of groundwater: hydrogeology and engineering. Hydrogeology Journal 10, pp. 121–142.

Boyd, E.S., Cummings, D.E. and Geesey, G.G. 2007. Mineralogy Influences Structure and Diversity of Bacterial Communities Associated with Geological Substrata in a Pristine Aquifer. Microbial Ecology 54, pp. 170–182.

Brown, J.C. 2007. Biological Treatments of Drinking Water. The Bridge 37, 4, pp. 30-36.

Campos, L.C., Su, M.F.J., Graham, N.J.D. and Smith, S.R. 2002. Biomass development in slow sand filters. Water Research 36, pp. 4543–4551.

Castro, F.D. and Tufenkji, N. 2008. Role of oxygen tension on the transport and retention of two pathogenic bacteria in saturated porous media. Environmental Science and Technology 42, pp. 9178–9183.

Chen, J., LeBoeuf, E.J., Dai, S. and Gu, B. 2003. Fluorescence spectroscopic studies of natural organic matter fractions. Chemosphere 50, pp. 639–647.

Collins, M. R., Eighmy, T.T., Fenstermacher, J.M. Jr. and Spanos, S.K. 1992. Removing Natural Organic Matter by Conventional Slow Sand Filtration. Journal American Water Works Association 85, 5, pp. 80-90.

Dash, R.R., Prakash, E.V.P.B., Kumar, P., Mehrotra, I., Sandhu, C. and Grischek, T. 2010. River bank filtration in Haridwar, India: removal of turbidity, organics and bacteria. Hydrogeology Journal 18, pp. 973‑983.

Díaz-Cruz, M.S. and Barceló, D. 2008. Trace organic chemicals contamination in ground water recharge. Chemosphere 72, pp. 333-342.

Eichler, S., Christen, R., Höltje, C., Westphal, P., Bötel, J., Brettar, I., Mehling, A. and Höfle, M.G. 2006. Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA- and DNA-based 16S rRNA gene fingerprinting. Applied and Environmental Microbiology 72, pp. 1858-1872.

Eleuterio, L. and Batista, J.R. 2010. Biodegradation studies and sequencing of microcystin-LR degrading bacteria isolated from a drinking water biofilter and a fresh water lake. Toxicon 55, pp. 1434–1442.

Farnsworth, C.E. and Hering, J.G. 2011. Inorganic Geochemistry and Redox Dynamics in Bank Filtration Settings. Environmental Science and Technology 45, pp. 5079–5087.

Fierer, N., Schimel, J.P. and Holden, P.A. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35, pp. 167–176.

Filella, M. 2009. Freshwaters: which NOM matters? Environmental Chemistry Letters 7, pp. 21-35.

Fischer, H. and Pusch, M. 1999. Use of the [^{14}C]leucine incorporation technique to measure bacterial production in river sediments and the epiphyton. Applied and Environmental Microbiology 64, pp. 4411-4418.

Flynn, T.M., Sanford, R.A. and Bethke, C.B. 2008. Attached and suspended microbial communities in a pristine confined aquifer. Water Resources Research 44, W07425.

Fox, P., Aboshanp, W. and Alsamadi, W. 2005. Analysis of soils to demonstrate sustained organic carbon removal during soil aquifer treatment. Journal of Environmental Quality 34, 1, pp. 156–163.

Frette, L., Jørgensen, N.O.G., Nybroe, O., del Giorgio, P.A. and Kroer, N. 2009. Effect of availability of nitrogen compounds on community structure of aquatic bacteria in model systems. Microbial Ecology 57, pp. 104–116.

Giebler, C. and Lueders, T. 2009. Microbial biodiversity in groundwater ecosystems. Freshwater Biology 54, pp. 649–677.

Gordon, C. and Toze, S. 2003. Influence of groundwater characteristics on the survival of enteric viruses. Journal of Applied Microbiology 95, pp. 536–544.

Groß-Wittke, A., Gunkel, G. and Hoffmann, A. 2010. Temperature effects on bank filtration: redox conditions and physical–chemical parameters of pore water at Lake Tegel, Berlin, Germany. Journal of Water and Climate Change 1, 1, pp. 55–66.

Grünheid, S., Amy, G. and Jekel, M. 2005. Removal of bulk dissolved organic carbon (DOC) and trace organic compounds by bank filtration and artificial recharge. Water Research 39, pp. 3219–3228.

Grützmacher, G., Böttcher, G., Chorus, I. and Bartel, H. 2002. Removal of microcystins by slow sand filtration. Environmental Toxicology 17, 4, pp. 386–394.

Grützmacher, G. and Reuleaux, M. 2011. Literature study on redox control for infiltration ponds and other subsurface systems. Berlin. Kompetenzzentrum Wasser Berlin gGmbH. 53 p.

Grützmacher, G., Wessel, G., Klitzke, S. and Chorus, I. 2010. Microcystin elimination during sediment contact. Environmental Science and Technology 44, 657-662.

Gunkel, G., Beulker, C., Hoffmann, A. and Kosmol, J. 2009. Fine particulate organic matter (FPOM) transport and processing in littoral interstices – use of fluorescent markers. Limnologica 39, pp. 185–199.

Heberer, T., Massmann, G., Fanck, B., Taute, T. and Dünnbier, U. 2008. Behaviour and redox sensitivity of antimicrobial residues during bank filtration. Chemosphere 73, pp. 451–460.

Helmisaari, H.-S., Derome, J., Hatva, T., Illmer, K., Kitunen, V., Lindroos, A.-J., Miettinen, I., Pääkkönen, J. and Reijonen, R. 2006. Artificial recharge in Finland through basin and sprinkling infiltration: soil processes, retention time and water quality. In: Recharge systems for protecting and enhancing groundwater resources. Proceedings of the 5th International Symposium on Management of Aquifer Recharge, ISMAR5, Berlin, Germany, 11-16 June, 2005. UNESCO IHP-VI Series on Groundwater 13, pp. 280-285.

Her, N., Amy, G., McKnight, D., Sohn, J. and Yoon, Y. 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC and fluorescence detection. Water Research 37, pp. 4295-4303.

Hiscock, K.M and Grischek T. 2002. Attenuation of groundwater pollution by bank filtration. Journal of Hydrology 266, pp. 139–144.

Hoffmann, A. and Gunkel, G. 2011a. Bank filtration in the sandy littoral zone of Lake Tegel (Berlin): structure and dynamics of the biological active filter zone and clogging processes. Limnologica 41, pp. 10-19.

Hoffmann, A. and Gunkel, G. 2011b. Carbon input, production and turnover in the interstices of Lake Tegel bank filtration site, Berlin, Germany. Limnologica 41, pp. 151–159.

Holst, T., Jørgensen, N.O.G., Jørgensen, C. and Johansen, A. 2003. Degradation of microcystin in sediments at oxic and anoxic, denitrifying conditions. Water Research 37, pp. 4748–4760.

Hoppe-Jones, C., Oldham, G. and Drewes, J.E. 2010. Attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems. Water research 44, pp. 4643-4659.

Hübner, U., Grummt, T. and Jekel, M. 2011. Optimisation of organic compound removal in artificial recharge systems by redox control and enhanced oxidation (OXIRED-1) – Final Report. Berlin. Kompetenzzentrum Wasser Berlin gGmbH. 64 p.

Huck, P.M. 1990. Measurement of biodegradable organic matter and bacterial growth potential in drinking water. Journal American Water Works Association 82, 7, pp. 78-86.

Huismans, L. and Wood, W.E. 1974. Slow sand filtration. World Health Organization. 122 p. Available at:
http://www.who.int/water_sanitation_health/publications/ssf9241540370.pdf

Humphreys, W.F. 2009. Hydrogeology and groundwater ecology: Does each inform the other? Hydrogeology Journal 17, pp. 5–21.

Karttunen, E. and Tuhkanen, T. 2003. Vesihuolto I. Helsinki, Suomen Rakennusinsinööriliitto RIL r.y., RIL 124-1. 314 p.

Keinänen, M.M., Korhonen, L.K., Lehtola, M.J., Miettinen, I.T., Martikainen, P.J., Vartiainen, T. and Suutari, M.H. 2002. The microbial community structure of drinking water biofilms can be affected by phosphorus availability. Applied and Environmental Microbiology 69, pp. 434-439.

Kirchman, D. 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods in Microbiology 30, pp. 227-237.

Kolehmainen R.E. 2008. Natural organic matter biodegradation and microbial community dynamics in artificial groundwater recharge. Doctoral thesis. Tampere. Tampere University of Technology, Department of Cehmistry and Bioengineering. Publication 781. 48 p.

Kolehmainen R.E., Korpela J.P., Münster U., Tuovinen O.H. and Puhakka J.A. 2009a. Extracellular enzyme activities and nutrient availability for bacteria during artificial groundwater recharge. Water Research 43, pp. 405-416.

Kolehmainen, R.E., Kortelainen, N.M., Langwaldt, J.H. and Puhakka, J.A. 2009b. Biodegradation of natural organic matter in long-term, continuous-flow experiments simulating artificial groundwater recharge for drinking water production. Journal of Environmental Quality 38, pp. 44-52.

Kolehmainen, R.E., Langwaldt, J.H. and Puhakka, J.A. 2007. Natural organic matter (NOM) removal and structural changes in the bacterial community during artificial groundwater recharge with humic lake water. Water Research 41, pp. 2715-2725.

Kolehmainen, R.E., Tiirola, M.A. and Puhakka, J.A. 2008. Spatial and temporal changes in Actinobacterial dominance in experimental artificial groundwater recharge. Water Research 42, pp. 4525-4537.

Kortelainen, N. 2011. Isotope tracing in groundwater applications. In: Nenonen, K. and Nurmi, P.A. (eds.). Geoscience for Society: 125th Anniversary Volume. Geological Survey of Finland, Special Paper 49, 358 p.

Kortelainen, N.M. and Karhu, J.A. 2006. Tracing the decomposition of dissolved organic carbon in artificial groundwater recharge using carbon isotope ratios. Applied Geochemistry 21, pp. 547 - 562.

Kortelainen, N.M. and Karhu, J.A. 2009. Geochemical and isotopic evolution of high-pH groundwater in a carbonate-bearing glacigenic aquifer, SW Finland. Hydrology Research 40, pp. 19-31.

Kortelainen, N.M., Korkeakoski, P.J. and Karhu, J.A. 2007. Origin of calcite in the glacigenic Virttaankangas complex. Bulletin of the Geological Society of Finland 79, pp. 5–15.

Kuster, M., Díaz-Cruz, S., Rosell, M., López de Alda, M. and Barceló, D. 2010. Fate of selected pesticides, estrogens, progestogens and volatile organic compounds during artificial aquifer recharge using surface waters. Chemosphere 79, 8, pp. 880-886.

Långmark, J. 2004. Biofilms and microbial barriers in drinking water treatment and distribution. Doctoral thesis. Stockholm. Royal Institute of Technology, Department of Land and Water Resources Engineering. TRITA-LWR PHD 1014. 67 p.

Långmark, J., Storey, M.V., Ashbolt, N.J. and Stenström, T.A. 2004. Artificial groundwater treatment: biofilm activity and organic carbon removal performance. Water Research 38, 3, pp. 740-748.

Laws, B.V., Dickenson, E.R.V., Johnson, T.A., Snyder, S.A. and Drewes, J.E. 2011. Attenuation of contaminants of emerging concern during surface-spreading aquifer recharge. Science of the Total Environment 409, pp. 1087–1094.

Leenheer, J.A. and Croué, J-P. 2003. Characterizing dissolved aquatic organic matter. Environmental Science and Technology 37, 18A-26A.

Lehtola, M.J., Miettinen, I.T., Vartiainen, T. and Martikainen, P.J. 2002. Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes. Water Research 36, pp. 3681–3690.

Li, X., Upadhyaya, G., Yuen, W., Brown, J., Morgenroth, E. and Raskin, L. 2010. Changes in the structure and function of microbial communities in drinking water treatment bioreactors upon addition of phosphorus. Applied and Environmental Microbiology 76, pp. 7473–7481.

Li, H., Xing, P., Chen, M., Bian, Y. and Wu, Q.L. 2011. Short-term bacterial community composition dynamics in response to accumulation and breakdown of Microcystis blooms. Water research 45, pp.1702-1710.

Maeng, S.K., Ameda, E., Sharma, S.K., Grützmacher, G. and Amy, G.L. 2010. Organic micropollutant removal from wastewater effluent-impacted drinking water sources during bank filtration and artificial recharge. Water Research 44, pp. 4003-4014.

Maeng, S.K., Sharma, S.K., Abel, C.D.T., Magic-Knezev, A. and Amy, G.L. 2011a. Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: Batch and column studies. Water Research 45, pp. 4722-4736.

Maeng, S.K., Sharma, S.K., Lekkerkerker-Teunissen, K. and Amy, G.L. 2011b. Occurrence and fate of bulk organic matter and pharmaceutically active compounds in managed aquifer recharge: a review. Water Research 45, pp. 3015-3033.

Mansell, J., Drewes, J.E. and Rauch, T. 2004. Removal mechanisms of endocrine disrupting compounds (steroids) during soil aquifer treatment. Water Science and Technology 50, 2, pp. 29–237.

Massmann, G., Greskowiak, J., Dünnbier, U., Zuehlke, S., Knappe, A. And Pekdeger, A. 2006. The impact of variable temperatures on the redox conditions and the behaviour of pharmaceutical residues during artificial recharge. Journal of Hydrology 328, pp. 141– 156.

Matilainen, A., Gjessing, E.T., Lahtinen, T., Hed, L., Bhatnagar, A. and Sillanpää, M. 2011. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. Chemosphere 83, pp. 1431–1442.

Metge, D.W., Harvey, R.W., Aiken, G.R., Anders, R., Lincoln, G. and Jasperse, J. 2010. Influence of organic carbon loading, sediment associated metal oxide content and sediment grain size distributions upon Cryptosporidium parvum removal during riverbank filtration operations, Sonoma County, CA. Water Research 44, pp. 1125-1137.

Miettinen, I.T., Vartiainen, T. and Martikainen, P.J. 1996. Bacterial enzyme activities in ground water during bank filtration of lake water. Water Research 30, 10, pp 2495-2501.

Myllykangas, T., Nissinen, T.K., Rantakokko, P., Martikainen, P.J. and Vartiainen, T. 2002. Molecular size fractions of treated aquatic humus. Water Research 36, pp. 3045–3053.

Onesios, K.M., Yu, J.T. and Bouwer, E.J. 2009. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 20, pp. 441–446.

Page, D. and Dillon, P. 2007. Measurement of the biodegradable fraction of dissolved organic matter relevant to water reclamation via aquifers. CSIRO, Water for a healthy country report series. 28 p.

Quanrud, D.M., Hafer, J., Karpiscak, M.M., Zhang, J., Lansey, K.E. and Arnold, R.G. 2003. Fate of organics during soil-aquifer treatment: sustainability of removals in the field. Water Research 37, 14, pp. 3401-3411.

Rauch, T. and Drewes, J.E. 2005. Quantifying biological organic carbon removal in groundwater recharge systems. Journal of Environmental Engineering 131, pp. 909-923.

Rauch-Williams, T., Hoppe-Jones, C. and Drewes. J.E. 2010. The role of organic matter in the removal of emerging trace organic chemicals during managed aquifer recharge. Water Research 44, pp. 449-460.

Ray, C., Grischek, T., Schubert, J., Wang, J.Z. and Speth, T.F. 2002. A perspective of riverbank filtration. Journal American Water Works Association 94, 4, pp. 149-160.

Rousk, J., Brookes, P.C., Glanville, H.C. and Jones, D.L. 2011. Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. Applied and Environmental Microbiology 77, pp. 2791-2795.

Schijven, J.F., Hoogenboezem, W., Hassanizadeh, S.M. and Peters, J.H. 1999. Modeling removal of bacteriophages MS2 and PRD1 by dune recharge at Castricum, Netherlands. Water Resources Research 35, 4, pp. 1101-1111.

Schink, B. 2004. Principles of anaerobic degradation of organic compounds. In: Jördening, H.-J. and Winter, J. (eds.). Environmental biotechnology. Concepts and Applications. Weinheim, Wiley-VCH. 464 p.

Schütz, K. 2008. Artificial groundwater recharge in forests: soil fauna and microbiology. Doctoral thesis. Basel. University of Basel, Faculty of Science. Thesis no: 8476. 137p.

Schütz, K., Kandeler, E., Nagel, P., Scheu, S. and Ruess, L. 2010. Functional microbial community response to nutrient pulses by artificial groundwater recharge practice in surface soils and subsoils. FEMS Microbiology Ecology 72, pp. 445–455.

SFS-EN 1484. 1997. Water analysis. Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC). Helsinki, Finnish Standards Association. 15 p.

SFS-EN ISO 6878. 2004. Water quality. Determination of phosphorus. Ammonium molybdate spectrometric method. Helsinki, Finnish Standards Association. 21 p.

SFS-EN ISO 11732. 2005. Water quality. Determination of ammonium nitrogen. Method by flow analysis (CFA and FIA) and spectrometric detection. Helsinki, Finnish Standards Association. 18 p.

SFS-EN ISO 13395. 1997. Water quality. Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection. Helsinki, Finnish Standards Association. 26 p.

Sharma, S.K. and Amy, G. 2010. Chapter 15: Natural Treatment Systems. In: Edzwald, J.K. (ed.). Water Quality and Treatment: A Handbook on Drinking Water. Sixth Edition. USA, American Water Works Association and McGraw Hill Inc. 233p.

Stevik, T.K., Aa, K., Ausland, G. and Hanssen, J.F. 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Research 38, pp. 1355–1367.

Toze, S. 2006. Reuse of effluent water - benefits and risks. Agricultural Water Management 80, pp. 147–159.

Toze, S., Bekele, E., Page, D., Sidhu, J. and Shackleton, M. 2010. Use of static Quantitative Microbial Risk Assessment to determine pathogen risks in an unconfined carbonate aquifer used for Managed Aquifer Recharge. Water Research 44, pp. 1038-1049.

Tufenkji, N., Ryan, J.N. and Elimelech M. 2002. The promise of bank filtration. Environmental Science and Technology 36, 21, pp. 422A-428A.

Turku Region Water Ltd. 2012. Artificial recharge system [www]. [cited 25.1.2012]. Available at: http://www.turunseudunvesi.fi/?pageKey=artificial_recharge_system

Turun Seudun Vesi Oy. 2011. Tekopohjavesijärjestelmän toiminta. Koekäyttövaiheen loppuraportti 2011. Turku, Turun Seudun Vesi Oy. 36 p.

Unger, M. and Collins, M.R. 2008. Assessing Escherichia coli removal in the schmutzdecke of slow-rate biofilters. Journal American Water Works Association 100, 12, pp. 60-73.

Urfer, D., Huck, M., Booth, S.D.J. and Coffey, B.M. 1997. Biological filtration for BOM and particle removal: a critical review. Journal American Water Works Association 89, 12, pp. 83-98.

World Health Organization. 1998. Guidelines for drinking-water quality. In: WHO. Health criteria and other supporting information, 2nd ed.; Addendum to Volume 2. Geneva, Switzerland, pp. 95–109.

Xue, S., Zhao, Q.L.,Wei, L.L. and Ren, N.Q. 2009. Behavior and characteristics of dissolved organic matter during column studies of soil aquifer treatment. Water research 43, pp. 499-507.

Xue, S., Zhao, Q.L.,Wei, L.L., Wang, L.N. and Liu, Z.G. 2007. Fate of secondary effluent dissolved organic matter during soil-aquifer treatment. Chinese Science Bulletin 52, 18, pp. 2496-2505.

Zhu, I. X., Getting T. and Bruce D. 2010. Review of biologically active filters in drinking water applications. Journal American Water Works Association 102, 12, pp. 67-77.